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A TEXT-BOOK

OF

CHEMICAL PHYSIOLOGY

AND

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BY

W. D. HALLIBURTON, M.D., B.Sc., M.R.C.P.

PROFESSOR OF PHYSIOLOGY AT KING'S COLLEGE, LONDON
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given my authority for any statements I have made, and I take this opportunity of thanking all those who have unwittingly, by their researches and writings, thus aided me in the production of this volume.

The illustrations are also culled from various sources, and I have to thank the following authors and publishers for the loan of blocks, or for permission to use certain illustrations: Dr. DUPRÉ, Prof. M. FOSTER, Mr. A. W. GERRARD, Mr. C. E. GROVES, Prof. MCKENDRICK, Prof. SCHÄFER, Dr. SCHUNCK, Mr. F. SUTTON; the Council of the Chemical Society; Messrs. CHURCHILL, Messrs. ENGELMANN of Leipzig, Messrs. MACMILLAN, Messrs. VIEWEG and SON of Brunswick, and Mr. HAWKSLEY.

The duty of reading the proof sheets has been greatly lightened for me by my friend Mr. C. J. MARTIN, B.Sc., Demonstrator of Physiology in King's College, who read the final revises, and I have to thank him for many valuable suggestions and alterations.

W. D. HALLIBURTON.

KING'S COLLEGE:
October 1, 1890.

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Errata

On p. 86, the formula for Cystin is wrongly given. It should be $C_3H_6NSO_2$. Its formula and constitution are correctly given on pp. 768, 769.

On p. 303, the formula for Spermine (Charcot's crystals) is incorrectly given. It should be C_2H_5N. This is given correctly on p. 563.

PART I

METHODS OF RESEARCH AND ANALYSIS

CHAPTER I

APPARATUS, REAGENTS, WEIGHTS AND MEASURES

THE methods of analysis of, and modes of examining the substances of which the body is composed vary a good deal from those ordinarily employed in chemistry. An examination of a living tissue by purely chemical means is well-nigh impossible, as the reagents used will be almost certain to destroy the life of the tissue in question. Hence some of the methods adopted by the physiologist differ markedly from those of the chemist : for instance, he may experiment upon himself or upon animals, giving certain foods or drugs by the mouth, and examining the result in the alimentary canal or their effect on the urine and other excretions ; again, he may attempt to imitate after death the conditions which obtain during life, and so conduct experiments upon ferment action, the changes in cells, and so on. The physiologist has to deal very largely with an important class of substances known as albuminous or proteid ; these require certain special methods of investigation which are but rarely used in the work of ordinary chemistry ; and then the physiologist avails himself of certain physical apparatus, such as the spectroscope, polariscope, &c., which give material help in the elucidation of chemical problems.

The reader must consult some special book on analytical chemistry for full details respecting analytical methods. The present chapters (Part I.) form a mere sketch of the chief operations performed in chemical investigation, those specially available for physiological work being dwelt on rather more fully.

WEIGHTS AND MEASURES

The weights and measures usually employed in science are those of the metric system ; but, as the practical physician still uses very largely English grains and ounces, we give here both systems.

Weights.

(English System.)

1 grain	= 0·0648 gramme
1 ounce = 437·5 grains	= 28·3495 grammes
1 lb. = 16 oz. = 7,000 grains	= 453·5925 „

REAGENTS AND APPARATUS

The reagents chiefly employed are distilled water, physiological saline solution (0·6 per cent. NaCl), alcohol, ether, glycerine, the mineral acids, acetic, oxalic and tannic acids, potash, soda, ammonia, lime water, baryta water, silver nitrate, barium chloride, lead acetate, copper sulphate, mercuric chloride, sodium chloride, carbonate and sulphate, magnesium sulphate, the carbonate, chloride, molybdate, oxalate, sulphide and sulphate of ammonium, &c. &c.

Normal Solutions used in analysis are of such a strength that 1 litre at 15° C. contains the hydrogen equivalent of the reagent in grammes. A normal solution of hydrochloric acid, for instance, will contain ($H=1+Cl=35{\cdot}5=36{\cdot}5$) 36·5 grammes of the acid in a litre of water. Or in the case of caustic soda, NaHO ($Na=23+H=1+O=16$), 40 grammes must be present in the litre. In the case of a bivalent substance the equivalent is half the atomic or molecular weight; thus a normal solution of oxalic acid which is dibasic ($C_2H_2O_4+H_2O=126$) contains 63 instead of 126 grammes dissolved in the litre.

Decinormal solutions are $\frac{1}{10}$, and centinormal solutions $\frac{1}{100}$ of the strength of normal solutions.

Empirical Standard Solutions are generally constructed so that 1 c.c. corresponds to 0·01 gramme (1 centigramme) of the substance to be estimated.

The Apparatus necessary consists of the ordinary appliances of the chemical laboratory: test-tubes, beakers, flasks, funnels, filters, dishes and crucibles, stirring rods, pestle and mortar, wash-bottles, retorts, pipettes, blow-pipe, balance, air and water-baths, exsiccators, measures, &c., and in addition certain forms of apparatus which are more fully described in subsequent chapters, such as microscope, spectroscope, polarimeter, dialyser, apparatus for gas analysis and for combustions, specific gravity bottles, urinometers, &c. &c. In addition, apparatus for the manufacture of carbonic acid, sulphuretted hydrogen, and other gases, is often needed.

CHAPTER II

ANALYTICAL METHODS

GRAVIMETRIC AND VOLUMETRIC ANALYSIS

GRAVIMETRIC analysis, or quantitative analysis by weight, consists in separating out the constituents of any compound in a pure state, or in the form of some new compound of known composition, and accurately weighing the products.

Volumetric processes are, as a rule, more quickly performed, and consist in submitting the substance to be estimated to certain characteristic reactions, employing for such reactions solutions of known strength, and from the volume of solution necessary for the production of such reaction determining the weight of the substance to be estimated.

Volumetric analysis consequently depends on the following conditions for its successful practice:—

1. A solution of the reagent, the chemical power of which is accurately known, called the 'standard solution.'
2. A graduated vessel from which portions of it may be accurately delivered, called the burette (fig. 1).
3. The reaction produced by the test solution with any given substance must either by itself, or by an indicator, be such that its termination is unmistakable to the eye, and thereby the quantity of the substance with which it is combined accurately determined.

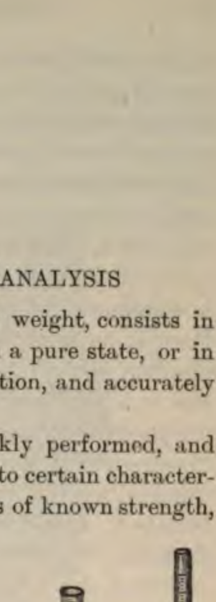

FIG. 1.—Two burettes on stand. (Sutton.)

The great advantage of volumetric processes is that the substance to be estimated need not be isolated in a pure condition, but the reaction chosen is generally one which is not interfered with by the presence of other substances.[1]

Suppose, for instance, one requires to know the amount of phosphoric acid in the urine; a measured amount of urine is rendered acid and boiled, and to it is added from a burette, a solution of known strength of uranium acetate (which for accuracy has been previously titrated[1] with a standard solution of sodium phosphate); the result of this is the formation of a compound of the uranium with the phosphoric acid, and this compound, called uranium phosphate, is insoluble in hot acid urine, and so a precipitate occurs. The precipitate, which is yellowish-white in colour, continues to form until all phosphoric acid is combined. When the precipitate ceases to form, one knows that there is no more phosphoric acid in solution, and if one adds more uranium acetate it will be left uncombined and free. One's object then is to add just sufficient of the standard solution to precipitate all the phosphoric acid. The volume of urine originally taken is known, the strength of the standard solution is known, and the amount of the standard solution that has been used can be ascertained by reading the burette.[2] It is, however, difficult to determine by the eye when precipitation is finished; an indicator is therefore used to detect any excess of the uranium salt; this is done by testing a drop of the mixture with a drop of potassium ferrocyanide on a white porcelain plate or testing-slab; this gives a reddish-brown precipitate with any uranium salt not combined with phosphoric acid. The appearance of such a brown precipitate indicates the end of the reaction.

---

[1] The above introductory sentences are taken almost verbatim from Sutton's [illegible] *Analysis.*

As an example, suppose the amount of urine taken was 50 c.c., and the amount of standard solution required for the appearance of the terminal reaction 24 c.c. The standard uranium solution used is of such a strength that 1 c.c. will exactly precipitate 0·005 gramme of phosphoric acid. 24 c.c. will precipitate 24 times 0·005=0·12 grammes. This is the amount of phosphoric acid in 50 c.c. of urine: the amount in 100 c.c. of urine will therefore be twice as great—c.c. 0·24 per cent.

This is an operation which can be completed in a few minutes, whereas if it had been necessary to separate out the phosphoric acid in a pure condition and weigh it, the processes would have extended over several days.

There are, however, certain substances to which a volumetric method cannot be applied, and it is then necessary to use the gravimetric method. The precautions to be used in such a method may be best illustrated by taking an example, and we may again select our example from the urine; in certain forms of morbid urine, proteid matter is present; it is often desirable to estimate it, and the best method for doing so accurately, is to precipitate it, and weigh the precipitate. Among the many precipitants of proteids, alcohol is on the whole the most convenient. A known volume of urine is evaporated to a small bulk, and if alkaline is rendered faintly acid; about ten times the amount of alcohol is added, and the mixture boiled. The precipitate is collected on a previously dried and weighed filter. The filter used must either contain no ash, or the amount of ash (i.e. mineral constituents) must be known.

---

[1] A titrated solution is one of which the strength has been accurately found by experiment. When a solution is directed to be titrated, the meaning is that it is to be quantitatively tested for the amount of pure substance it contains by the help of standard or previously titrated solutions.

[2] It is usual to note the position of the lowest point of the curved surface (meniscus) of the fluid in a burette. Sometimes accuracy is obtained by using a glass float which rises and falls with the fluid in the burette without wavering; this has a horizontal line drawn on it, and the coincidence of this line with the graduation mark on the burette is accepted as the true reading.

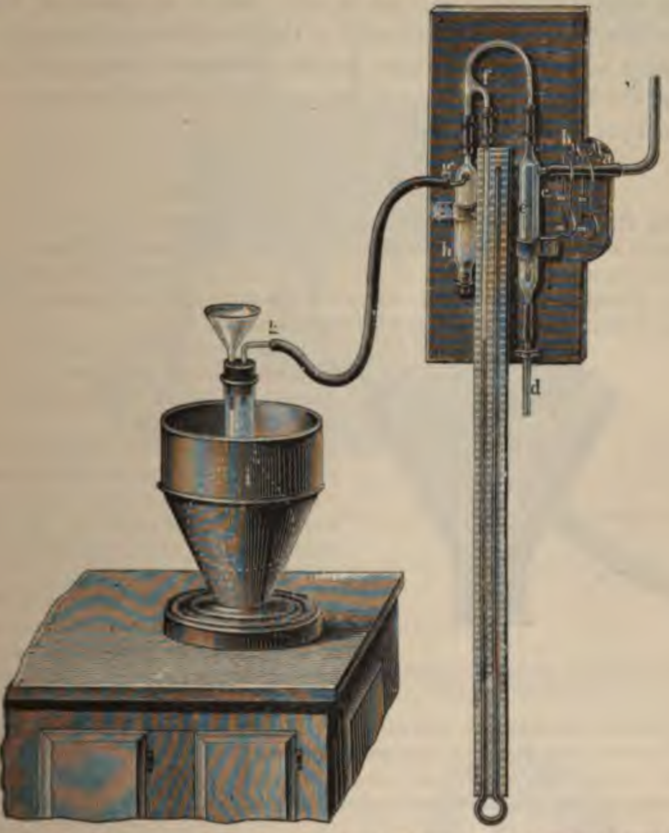

FIG. 2.—Sprengel's filter pump. Water enters by tube *w*, and escapes by *d*, drawing air with it from the rest of the apparatus, so producing a partial vacuum in the flask *t*, into which filtration is performed. The gauge *f* indicates the pressure, and the clips *a* and *b* regulate the rate of flow of water. (Gscheidlen.)

The precipitate is then thoroughly washed with alcohol and ether to remove all the other constituents of the urine; the filter, with the precipitate on it, is dried at 110° C., cooled in an exsiccator, and again weighed. The increase in weight is the amount of albuminous substance in the volume of urine originally taken. Proteid, however, carries down with it a certain amount of ash; this is estimated as follows: A crucible is dried and weighed; in this the filter and precipitate are carefully burnt, until ash only remains, allowance being made for the ash of the filter, this amount of ash must be deducted from that of the proteid previously found.

FILTRATION

The filter should be smaller than the funnel into which it is inserted, and generally should be moistened with water before being used. Filtration may be hastened by the use of ribbed filters; hot liquids also filter more quickly than cold. Filtration under pressure may be accomplished by using one of the many forms of filter pump of which one is here figured (fig. 2).[1]

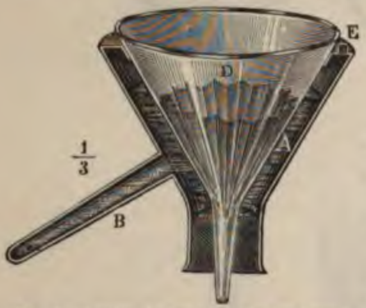

FIG. 3.—Sectional view of hot water funnel. (Gschleidlen.)

In order to keep a liquid hot during filtration, the ordinary glass funnel is enclosed in a hollow copper funnel filled with hot water (fig. 3).

In the case of fine precipitates, a small portion of the precipitate may at first pass through the filter; soon, however, the larger pores of the paper become plugged, and the portions, which first passed through, must be returned to the filter.

With voluminous, dense, or gelatinous deposits the separation of the greater part of the precipitate may be accomplished by filtering through muslin or linen.

Precipitates are now often collected on asbestos filters; these are readily made by perforating the bottom of a platinum crucible with fine holes and filling up the bottom of the crucible with finely divided asbestos. They are advantageous, as they can be easily dried and weighed, are permanent, and where incineration is necessary are free from ash.

WASHING PRECIPITATES

Precipitates may be washed on the filter; it is best to use a wash-bottle, and care must be taken that the wash-liquid penetrates to every part of the precipitate, which may be gently disturbed for the purpose with a glass rod.

Washing by decantation is only applicable to precipitates that are heavy and subside readily; the precipitate is well mixed with the wash-liquid and allowed to settle; the wash-liquid is then poured, syphoned, or pipetted off, more liquid added, and the process repeated as often as necessary.

---

[1] Simple forms of filter pump can now be purchased for a few shillings, and can be attached to any ordinary water-tap.

DRYING

A water-oven at the temperature of 100°, or, better still, an air-bath 10° or 20° higher, and the temperature kept constant by a gas regulator, may be used for drying filter-papers and organic substances generally. Crucibles may be readily and quickly dried by holding them with tongs for a few seconds in a Bunsen flame.

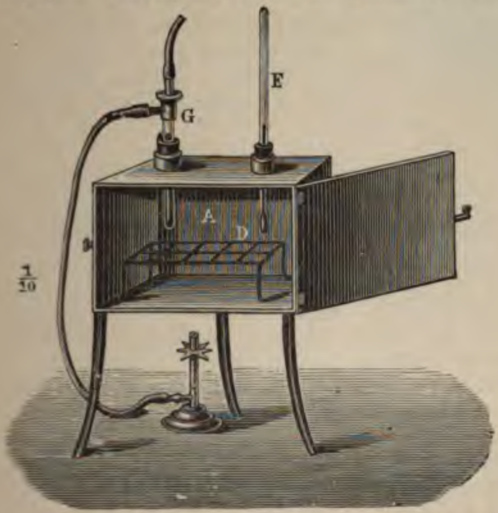

FIG. 4.—Hot-air oven with gas regulator (G). (Gschleidlen.)

A flask is dried by warming it and then sucking the air from the interior with a long glass tube dipping into it.

COOLING AFTER DRYING

Substances must not be weighed hot, otherwise air currents are set up which disturb a delicate balance. They must not be allowed to dry in the air, or they (especially if hygroscopic) become moist again. They are generally cooled in an exsiccator—a closed glass vessel containing a tray of sulphuric acid. *See* figure 5.

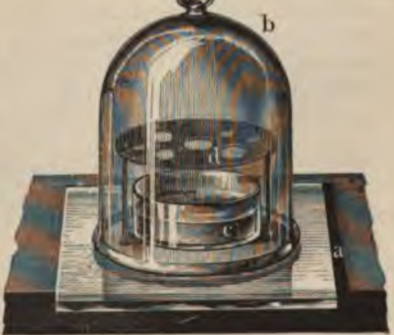

FIG. 5.—An exsiccator. (Gschleidlen.)

A filter is usually allowed to cool and is weighed between two watch-glasses clipped together, or in a thin wide-mouthed glass bottle. The bottle or watch-glasses, however, must be dry, and cooled before weighing, in an exsiccator. After weighing any substance that has been dried, it is again heated to 110° for some hours, cooled and weighed as before; the process being repeated until two successive weighings give the same result—that is, till there is no more loss of weight from evaporation of water. This is called weighing to constant weight. Exsiccators may be used for drying *in vacuo* at a low temperature such substances as would be injured by the application of heat. In such cases the top of the bell jar of the exsiccator is connected by a tube to an air-pump. The tube should be fitted with a stop-cock. The air is thus exhausted either by an ordinary air-pump or by a water air-pump constructed on the same principle as that already described (fig. 2). When the vacuum is as complete as possible the stop-cock just mentioned should be closed and the pump can be detached. Moisture rapidly passes off from the substance which is to be dried, and is absorbed by the sulphuric acid.

INCINERATION

The substance to be incinerated must be dry and must not touch the side of the crucible more than is absolutely necessary. A crucible of known weight is placed upon a triangle over a Bunsen flame and at first heated very cautiously, or the contents are apt to froth and be partially lost. The heat is gradually increased, and ultimately the flame is allowed to surround the crucible, which should be tilted. The process, which is a long one with porcelain crucibles, is allowed to continue till the ash is white, when it may be cooled and weighed.

Rose's method is better than the preceding, and is as follows:—

The dry substance is carefully carbonised in a crucible over a Bunsen flame. After cooling the contents are heated with distilled water again and again to dissolve all soluble salts. The hot aqueous extracts are mixed and filtered through a small filter of known ash.[1] The insoluble matters together with this small filter are dried at 110° and ignited at a red heat; when the residue is white the crucible is cooled and weighed; this gives, subtracting the weights of the crucible and filter ash, the weight of the insoluble salts. Either in the same crucible or in a separate one the aqueous extract is evaporated to dryness, dried at 110°, and ignited at a red heat; the crucible is then cooled and weighed; the increase in weight is the amount of soluble salts.

EVAPORATION

The usual temperature employed is 100°, which is most easily obtained with a water-bath; for lower temperatures, a water-bath is kept at a constant temperature by a gas regulator.

Ethereal or alcoholic solutions must never be evaporated over a naked flame, but over a water-bath. In heating glass vessels it is also advisable to interpose a flat iron plate, or a piece of wire gauze, or asbestos cardboard, or a sand-bath between the glass and the flame.

BOILING

The boiling point of a liquid is that temperature at which the liquid becomes no hotter, but at which any further heat is used up in converting the liquid into vapour. If the liquid, however, be enclosed in a vessel so that no vapour can escape, its temperature will continue to rise. Hence it is possible to heat aqueous solutions above 100° C. by enclosing them in sealed tubes or a Papin's digester, and placing these in a liquid such as oil, which boils at a higher temperature than water.

---

[1] The ash of a filter may be ascertained by incinerating, say, 12 similar filters, weighing the ash, and obtaining the average by dividing by 12.

Sometimes one requires to heat a liquid for a long time without its losing much of its bulk; a long glass tube or a condenser is then attached by a cork with a hole in it to the neck of the flask, the vapour condenses in the tube and runs back into the flask.

DISTILLATION

Some substances are much more volatile—that is, boil at a lower temperature—than others. Advantage is taken of this to separate such substances by a process of distillation.

A distillation apparatus consists essentially of a boiler and a condenser. The boiler may be a flask or a retort closed with a cork through which a tube passes; the tube leads to the condenser, which in the form commonly used (Liebig's) consists of a long tube surrounded by an outer tube; cold water is made to circulate between the two tubes, and thus the vapour which passes along the inner tube is condensed, and the fluid so formed is collected at its far end.

A thermometer is fixed into the retort through the cork at its summit; fractional distillation consists in collecting the substances in separate vessels that distil over at different temperatures.

DIALYSIS

If a solution of albuminous, gelatinous, or mucilaginous substances, mixed with saline and crystalline substances be placed in a dialyser, in distilled water, it will be found that the crystalline substances pass through the parchment membrane into the water, while the proteid or gelatinous substances remain in the dialyser. The substances which pass through membranes in this way are generally crystallisable, and were termed *crystalloids* by Thomas Graham. The substances which are indiffusible are generally non-crystalline (a striking excep-

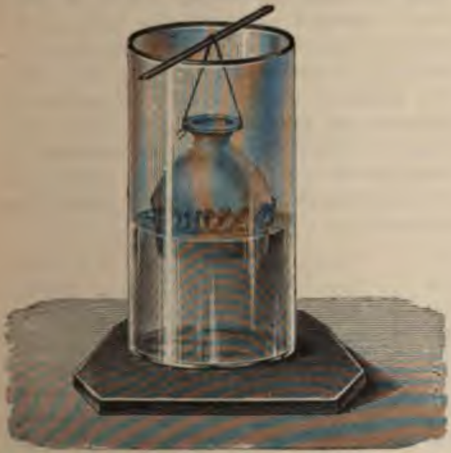

Fig. 6.—Dialyser. The lower opening of the bell jar suspended in water is tightly covered with parchment paper. The fluid to be dialysed is placed within this vessel, the crystalloids pass out into the distilled water outside, through the parchment paper.

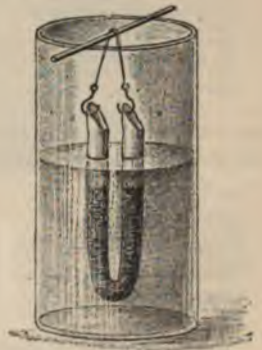

Fig. 7.—In this form of dialyser the substance to be dialysed is placed within the piece of tubing suspended in the larger vessel of water. The tubing is made of parchment paper.

tion to this rule, however, is hæmoglobin), and were termed ***colloids*** by Graham. The distinction is generally stated to be due to the large size of the molecules of colloid materials, rendering them unable to pass through the membrane.

The forms of dialyser employed are depicted in the accompanying figures. That in fig. 7 is the more convenient, as by its use a larger surface is exposed to the action of water, and so the time necessary for diffusion is lessened.

In dialysing to get rid of salts from organic material, as long a time as four to seven days is generally necessary; different salts vary a good deal as to the rate at which they pass out. The distilled water in the outer vessel should be changed frequently; or, better still, a stream of water running from the tap should be kept continually flowing for three to four days, and then distilled water be used for the last few days of the operation; this should be contained in a large vessel, and changed three or four times a day.

Occasionally one dialyses into other liquids than water; *e.g.* in Haycraft's method of estimating urea in blood and similar liquids, one dialyses into alcohol.

Taking distilled water as a standard, the rate and amount of diffusibility may be measured by an endosmometer. It will be found that a constant relation exists between the weight of water which passes in one direction, and that of salt which passes in the other. The weight of water necessary to replace by diffusion one gramme of the dissolved substance is called the ***endosmotic equivalent*** of that substance, which in its turn depends on the nature of the substance and its concentration. The following table gives the endosmotic equivalent of certain materials :—

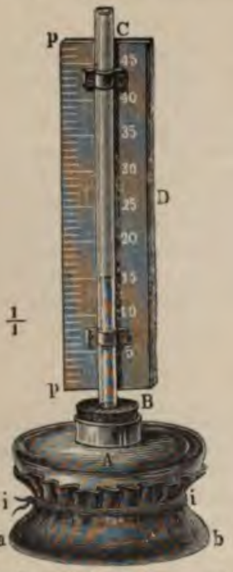

FIG. 8.—Dutrochet's Endosmometer. A, glass cylinder constructed so that an organic membrane (piece of bladder) *a*, *b*, can be tied over the lower end by the ligature *i*, *i*. The tube C is passed through a cork B, fitting tightly into the upper constricted end of the cylinder. D a scale attached to C divided into millimetres. (Gscheidlen.)

Sodium chloride . . .	4·0	Sulphuric acid . . .	0·3
Sodium sulphate . . .	11·0	Caustic potash . . .	200·3
Potassium sulphate . .	12·0	Alcohol	4·3
Magnesium sulphate . .	11·5	Sugar	7·2
Copper sulphate . . .	9·5		

Thus 4 grammes of water would pass through the membrane into the endosmometer for 1 gramme of sodium chloride, 11 for 1 gramme of sodium sulphate, and so on. Sometimes negative osmosis occurs, *i.e.* more of the substance passes out of the osmometer than water passes in; this is the case with acids. The rate of osmosis increases with the concentration of the substance with the temperature of the liquids used. There is also no doubt that the nature of the membrane affects osmotic action; different varieties of dead membrane affect the rate of osmosis; the osmosis that occurs in living membranes is also no doubt very different again, but is a difficult subject to investigate experimentally. A living membrane is not fixed or stable, but is constantly undergoing processes of building up and breaking down.[1] Thus the discussion whether the formation of lymph is due to filtration or diffusion of the blood plasma through the vessel walls has not yet received a satisfactory answer.[2] The question is still further complicated in the living body by the fact that the fluids on the two sides of any membrane are almost invariably at different pressures; and in addition it is possible that there may be some kind of attractive influence exerted by the tissues themselves, analogous to the selective activity of secreting cells.

DETERMINATION OF SPECIFIC GRAVITY

The specific gravity of liquids is usually ascertained by a hydrometer; and these instruments adapted for the range of specific gravities in urine and milk, are termed urinometers and lactometers respectively.

But when the specific gravity must be determined with greater accuracy, a small light flask of known weight, called a pycnometer or specific gravity bottle, is employed. This is fitted with a stopper, through which a capillary canal passes,

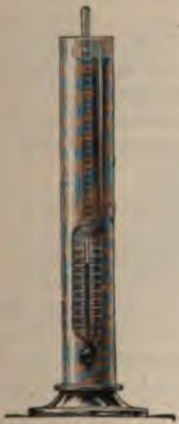

FIG. 9.—Urinometer floating in urine in a testing glass.

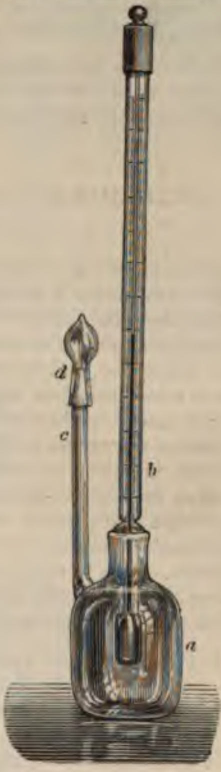

FIG. 10.—Geissler's specific gravity bottle. *a* is a light flask, *b* an accurate thermometer; *c* is a tube connected with *a*, through which fluid escapes when the thermometer is inserted in the bottle; *d* a cap to fit on to the top of *c*.

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[1] In a recent paper, Prof. Waymouth Reid (*Brit. Med. Journ.* vol. i. 1890, p. 165) brings out very clearly the difference as regards diffusion between dead and living membranes. The membrane he experimented with was the skin of the frog. As he points out, we have doubtless in a living membrane to deal with an absorptive force dependent on protoplasmic activity, and comparable to the excretive force of a gland cell. This is excited especially to make osmosis take place more readily in one direction than in the other; in the case of the frog's skin from without in.

[2] A discussion on this subject will be found in Foster's *Physiology*, vol. ii. p. 503, 5th edit., 1889.

and contains when filled a known weight of water (25–30 grammes) at 15° C. Some pycnometers are fitted with thermometers, used at the temperature of the air, whatever it happens to be, and then the weight of water in it calculated from the table on p. 5.

The bottle is filled with the liquid the specific gravity of which is to be determined; it is weighed, and the specific gravity or density obtained by the formula: sp. gr. $=\frac{w}{w'}$: w = the weight of the liquid, w' = the weight of the water.

The result is then obtained in comparison with water, which is taken as unity. In medical work it is often found more convenient to take water at 1000; a urine of specific gravity 1020 means one which is 1·02 times heavier than water bulk for bulk.

DETERMINATION OF REACTION. ALKALIMETRY. ACIDIMETRY

Litmus papers, or a neutral litmus solution, are usually employed to determine whether a substance is neutral, acid, or alkaline.

Neutral substances have no effect on either red or blue litmus, but in presence of organic materials a neutral solution will often turn delicate glazed blue litmus papers faintly red, and red ones faintly blue.

Acid substances turn blue litmus red. In the case of volatile acid, the red colour passes off as the acid evaporates.

Alkaline substances turn red litmus blue. At night it is best to examine the transition in colour by monochromatic (sodium light); the red colour appears colourless, the blue is blackish. In measuring the amount of acidity or alkalinity of a solution it is titrated with a standard solution of acid or alkali respectively; the indicator of the end of the process being the change in colour produced in the litmus. It is, in fact, a simple example of the volumetric method. Recently, however, more delicate indicators than litmus have been employed, and the following is a list of the principal ones:—

1. Methyl orange, 1 gramme dissolved in a litre of water. This is only applicable to titration with mineral acids; it is not affected by carbonic or sulphydric acids in the cold. It is an admirable indicator for ammonia and its salts. The colour given is pink with acid, yellow with alkali.
2. Phenacetolin, 2 grammes dissolved in 1 litre of alcohol. The solution is dark brown; it gives a scarcely perceptible yellow with caustic soda or potash; with ammonia and the normal alkaline carbonates a dark pink; with the bicarbonates a brighter pink; and with the mineral acids a golden yellow.
3. Phenolphthalein, 1 gramme in 1 litre of 50 per cent. alcohol. A few drops of the indicator show no colour in the ordinary volumes of neutral or acid liquids, the faintest excess of caustic alkalis gives a sudden change to purple-red. It possesses the advantage of great delicacy, but the disadvantage of being useless for the titration of free ammonia or its compounds.
4. Rosolic acid, 2 grammes in 1 litre of 50 per cent. alcohol. Its colour is pale yellow unaffected by acids, but turning to violet-red with alkalis. It is not reliable for organic acids.
5. Lacmoid. This is prepared from resorcin, and behaves like litmus. Lacmoid paper is also prepared. Lacmoid, rosolic acid, phenacetolin and

phenolphthalein are capable of showing change of colour with $\frac{1}{5}$ of the quantity of acid or alkali necessary in the case of methyl orange or litmus.[1]

The normal solutions most frequently used in estimating acidity or alkalinity are those of sodium carbonate (53 grms. Na_2CO_3 per litre), potassium carbonate (69 grms. K_2CO_3 per litre), sulphuric acid (49 grms. H_2SO_4 per litre), oxalic acid (63 grms. of $C_2H_2O_4.2H_2O$, or 45 grms. of $C_2H_2O_4$ per litre), hydrochloric acid (36·37 grms. HCl per litre), caustic soda or potash (40 grms. NaHO, or 56 grms. KHO per litre), and semi-normal ammonia[2] (8·5 grms. NH_3 per litre).

100 c.c. of any of the acid solutions exactly neutralise the same volume of any of the alkaline solutions, except in the case of semi-normal ammonia, which requires only half the quantity of acid.

THE CENTRIFUGAL MACHINE

The separation of precipitates too fine to filter off, of corpuscles from serum, of cream from milk, &c. &c., may be facilitated by subjecting the fluid to the action of a centrifugal machine (see figure). The liquid is placed in tubes at the margin of a horizontal rotating disc, worked at a high rate of speed by machinery (1000 revolutions per minute). The tubes fly into the horizontal direction, and the heavy particles settle to the far end of the tube; the upper fluid can then be decanted or pipetted off. The time that this takes varies with the relative densities of the substances to be separated. Serum and blood corpuscles are usually separable by this means after about 30 to 60 minutes' whirling.

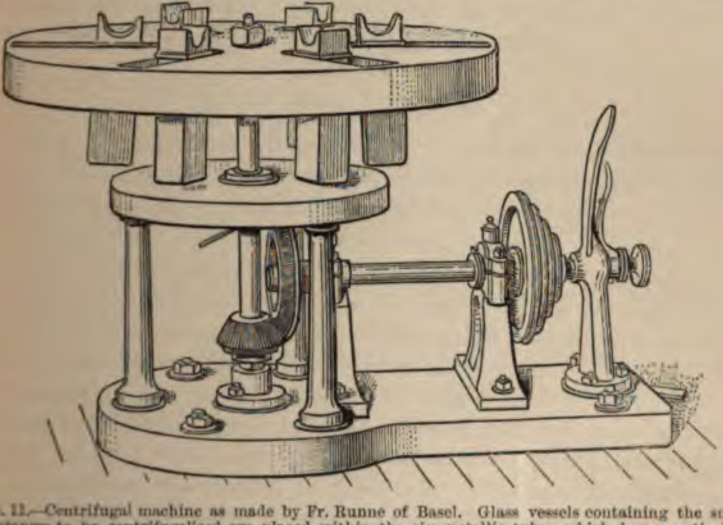

FIG. 11.—Centrifugal machine as made by Fr. Runne of Basel. Glass vessels containing the substances to be centrifugalised are placed within the six metallic tubes which hang vertically while the disc is at rest: when the machinery is set going they fly out into the horizontal position. A water motor is a very convenient motive power for these instruments.

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[1] For full particulars see Thompson, *Chem. News*, vol. 47, pp. 123, 185, vol. 48, pp. 32, 119.

[2] It is unsafe to use normal ammonia.

HEAT COAGULATION AND SATURATION WITH SALTS

are processes used especially in connection with the proteids under which they are fully described.

DETERMINATION OF RELATION OF SOLIDS AND WATER IN ANY SUBSTANCE

If the substance is liquid, a weighed quantity is evaporated to dryness in a weighed crucible or capsule on a water-bath, and the residue is then thoroughly dried to constant weight in an air-bath (110° C.). If the substance is solid it is finely divided and weighed in a weighed crucible, then dried to constant weight at 110° C.

In each case the total loss of weight is the amount of water, the weight of the residue is the amount of total solids. These numbers should be, for convenience sake, calculated out as percentages. The relation of organic to inorganic solids may then be somewhat roughly determined by incinerating the residue; the ash remaining after the burning of the organic substance gives the weight of inorganic, the loss of weight on ignition that of the organic substances.

CHAPTER III

ULTIMATE ANALYSIS OF ORGANIC COMPOUNDS

THE ultimate analysis of organic compounds has for its object the determination of the elements contained in them. A small number of organic compounds consist of carbon and hydrogen, the greater number contain carbon, hydrogen, and oxygen, most of the rest carbon, hydrogen, oxygen, and nitrogen, and a small number sulphur, and a smaller number still sulphur and phosphorus in addition.

The same method of analysis applies to compounds whether they contain oxygen or not ; it is, however, necessary before commencing a quantitative analysis that the operator should know positively whether nitrogen, sulphur, or phosphorus is present or absent.

TESTS FOR NITROGEN

1. Burn the substance ; if it contains a tolerably large amount of nitrogen, the characteristic odour of burnt hair or feathers is given off. If the smell is distinctly perceptible no further test is necessary, but if not, a more delicate method must be adopted.

2. The substance is mixed with potassium hydrate in powder, or with soda-lime, and the mixture heated in a test-tube. If the substance contains nitrogen, ammonia will be evolved, which may be detected by its odour, reaction, and fuming with hydrochloric acid ; or the products of combustion may be conducted into dilute hydrochloric acid ; evaporate the acid on the water-bath, dissolve the residue in water, mix the solution with platinum tetrachloride, evaporate nearly to dryness, and treat the residue with alcohol. If the residue dissolves without leaving any double chloride of ammonium and platinum, the substance may be considered free from nitrogen.

TESTS FOR SULPHUR

1. Solids are fused with about 12 parts of pure potassium hydrate and 6 of potassium nitrate ; the mass is allowed to cool, dissolved in water, acidified with hydrochloric acid, and tested for sulphates with barium chloride. Care must be taken that the reagents used are free from sulphuric acid.

2. Liquids are treated with fuming nitric acid, or a mixture of nitric acid and potassium chlorate, at first in the cold, and finally with the application of heat; the solution is evaporated nearly to dryness, diluted, filtered, if necessary, and then tested for sulphates.

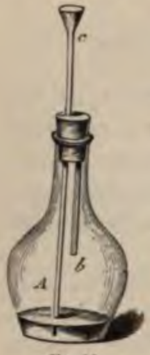

FIG. 12.

3. The following test serves to detect sulphur in organic compounds in the unoxidised state only. The substance is boiled with a strong solution of potassium hydrate, and evaporated nearly to dryness. The residue is dissolved in a little water, poured into a small flask A, which is then loosely corked. Through the cork a funnel tube *c* passes, which is allowed to dip into the fluid at the bottom. A slip of paper *b*, moistened with lead acetate and then with a few drops of ammonium carbonate, is allowed to hang down the neck of the flask; dilute sulphuric acid is poured down the funnel; if sulphur is present the slip of paper is turned brown from the action of the sulphuretted hydrogen which is evolved; or the sulphide of potassium may be detected by a solution of lead oxide in soda, which is turned black or brown.

TESTS FOR PHOSPHORUS

The methods 1 and 2 just described in testing for sulphur may also be employed for phosphorus. The solution obtained is examined for phosphates, either by a mixture of magnesium sulphate, ammonium chloride and ammonia, which gives a white precipitate, or preferably by the yellow crystalline precipitate given by a nitric acid solution of ammonium molybdate. If method 2 is used the greater part of the nitric acid must be first removed by evaporation.

QUANTITATIVE ANALYSIS OF SUBSTANCES CONSISTING OF CARBON AND HYDROGEN, OR OF CARBON, HYDROGEN, AND OXYGEN

The principle first proposed by Liebig for the analysis of these compounds was as follows. The substance is burnt and carbonic acid and water are formed; these products are separated from one another and weighed; the carbon is calculated from the weight of carbonic acid, the hydrogen from the amount of water. If the sum of the carbon and hydrogen is equal to that of the original substance, that substance contains no oxygen; if it is less than the weight of the substance, the difference expresses the amount of oxygen present. Methods have been proposed for the direct estimation of oxygen, but the oxygen is generally obtained by difference.

The combustion is effected either by igniting the organic substance with oxygenated substances that part readily with their oxygen (copper oxide, lead chromate, &c.), or in the case of difficultly combustible bodies, free oxygen is used also. Volatile substances require special precautions described in detail in works on organic analysis.[1]

The usual method adopted for solid, readily combustible, non-volatile substances such as sugar or starch is that of combustion with oxide of copper. The substance to be analysed is finely pulverised, dried, and weighed. A combustion tube is carefully dried and half filled with warm oxide of copper, which has been intimately mixed with the substance to be analysed, in a mortar; the tube is then filled up with pure oxide of copper, and a plug of copper turnings; the tube is then surrounded with hot sand and the air slowly pumped out from it, a calcium chloride tube being interposed between the combustion tube and the air pump. Air is then readmitted through the calcium chloride tube into the combustion tube, being dried by the calcium chloride as it passes in; the tube is again exhausted, fresh air admitted, and the operation repeated 10 or 12 times to ensure the removal of all moisture which the oxide of copper may have absorbed during the process of mixing. The chloride of calcium tube is then replaced by another which has been accurately weighed, and to the far end of this weighed potash bulbs are fixed by an india-rubber tube. The apparatus should be perfectly air tight; the combustion tube should be placed in a furnace so that its mouth projects 3 or 4 centimetres beyond it; there are many forms of combustion furnace made. The oxide of copper, not mixed with the substance to be analysed, is heated first; when this is red hot, the mixture is then heated. The air is driven out, and so are the carbonic acid and water produced by combustion; the water is detained by the chloride of calcium, the carbonic acid by the potash in the bulbs; and the increase of weight in these portions of the apparatus gives respectively the weight of water and carbonic acid.

DETERMINATION OF THE CARBON AND HYDROGEN IN NITROGENOUS SUBSTANCES

The same general method is employed, but without modification would give too much carbon, as the potash bulbs would retain not only the carbonic acid, but also the nitrous acid, and a small quantity of the nitric oxide formed. This defect may be avoided by the exclusive use of oxide of copper as oxidising agent, by a very intimate mixing of the substance with the oxide, by burning very slowly, and by selecting a combustion tube about 12–15 cm. longer than usual, filling this in the ordinary way, but finishing with a loose layer about 9–12 cm. long of clean fine copper turnings or a spiral of copper wire or foil ; this metal when red hot acts by decomposing all oxides of nitrogen into oxygen with which it combines, and nitrogen which passes on unaffected into the atmosphere.

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[1] I have found Fresenius translated by P. E. Groves, F.R.S., a most useful handbook. To it I am indebted for many of the methods given above.

DETERMINATION OF THE NITROGEN IN ORGANIC COMPOUNDS

1. Dumas' Method.—A long combustion tube, 70–80 cm. in length, is sealed at its far end like a test-tube. Pure dry bicarbonate of soda is first introduced so as to form a layer 12–15 cm. long : then the weighed substance intimately mixed with copper oxide, then the oxide with which the mortar in which the mixing was performed was rinsed out ; then a layer of pure oxide, and lastly a layer of copper wire, foil, or turnings about 15 cm. long. The tube is placed on a furnace, its open end closed by a cork through which a tube passes ; the tube is bent and leads to a trough containing mercury. About 6 cm. of the far end of the tube is then heated, the rest being protected by a screen ; the bicarbonate is decomposed and the carbonic acid propels the air before it, expelling it from the tube. After some time the end of the delivery tube is dipped under the mercury and a test-tube filled with potash solution inverted over it. If the gas bubbles are completely absorbed by the potash, all air must have been expelled from the tube ; if not, the evolution of gas is continued till this desired point is obtained. The actual combustion is then commenced. A graduated cylinder half filled with mercury and half with potash is inverted over the delivery tube. The combustion tube is heated, commencing with the copper foil, and gradually all the burners are lighted till those under the second half of the bicarbonate are all in full blaze. The whole of the gases evolved are driven on by the carbonic acid so evolved, the oxides of nitrogen are reduced by the metallic copper, and nitrogen in a moist state alone collects in the cylinder, the carbonic acid being absorbed by the potash. The weight of nitrogen is calculated from its volume, corrections to normal pressure and temperature being made, due regard being paid also to the tension of aqueous vapour (*see* further Gas Analysis).

2. Method of Varrentrapp and Will.—This method is founded on the same principle as the test for nitrogen already described (p. 19, test 2). The substance to be analysed is reduced to the finest powder, dried and weighed. It is mixed with soda-lime. A combustion tube is filled so that the mixture lies between two portions of pure soda-lime, and is then plugged loosely with asbestos, and finally with a cork which is perforated, allowing the tube and a bulb apparatus containing hydrochloric acid to be put in connection with one another. The tube is gradually heated, commencing at the fore part and progressing slowly towards the closed end. The nitrogen present is all thus converted into ammonia which is absorbed by the hydrochloric acid in the bulbs. The ammonia present is estimated by adding platinic chloride and weighing the ammonium platinum chloride which is thus precipitated.

3. **Kjeldahl's Method.**—The difficult and lengthy operations involved in the two methods just described, and present also in the many modifications of them which chemists have proposed from time to time, are however now unnecessary, as the simple and accurate method of Kjeldahl[1] has very largely replaced them.

I take the following account of the method with the modifications proposed by Warington[2] from Sutton's Volumetric Analysis.[3] I have myself frequently used the method, but it hardly needs now my testimony to its usefulness, as it has been so widely praised and so much adopted by others.

From 0·1 to 1 gramme of the dry powdered substance is put into a boiling flask holding about 100–120 c.c. The acid used for the destruction of the organic material is made by mixing 200 c.c. pure oil of vitriol, 50 c.c. Nordhausen oil of vitriol, and 2 grammes of phosphoric acid in sticks; all these must of course be free from ammonia. 10–20 c.c. of this mixture is poured over the substance in the flask and heated on wire gauze over a small Bunsen flame. The temperature must be kept below boiling; with prolonged heating the organic matter is gradually destroyed, and the liquid becomes clear and quiet. The nitrogen originally present is thus converted into ammonia, and this may be hastened by adding to the liquid very minute pinches of pure potassium permanganate. A violent commotion takes place with every addition, but there is no fear of any ammonia being lost. The operation is ended when the mixture becomes permanently greenish, and moderate heat is continued for a few minutes more. The flask is cooled, some water added, and the contents washed out into a large flask of 700 c.c. capacity with as little water as possible. It is then made alkaline with excess of either pure caustic soda or potash solution (sp. gr. 1·3). A little metallic zinc is added to prevent bumping during the subsequent distillation. The flask is then closed with a perforated caoutchouc stopper, through which passes an upright tube with two bulbs about an inch in diameter blown upon it; these arrest and carry back any spray of soda from the liquid. The tube above the bulbs is bent over and connected to a condenser, and the delivery end of the condenser leads into a flask containing a measured excess of standard acid.[1] The mixture in the flask is then distilled, the ammonia passes over into the acid. The amount of acidity is then determined in the distillate by titration with standard potash or soda, methyl orange being used as the indicator of the end of the reaction.

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[1] *Zeit. Anal. Chem.* xxii. p. 366.

[2] *Chem. News*, lii. p. 162.

[3] pp. 68–70.

Example.—Suppose 0·15 gramme of a nitrogenous substance is taken, treated with acid, neutralised and the ammonia distilled over and received by 100 c.c. of a decinormal solution of hydrochloric acid (=10 c.c. normal acid). The distillate is then titrated with decinormal soda and it is found that the neutral point is reached when 60 c.c. of the decinormal soda have been added. The other 40 c.c. must therefore have been neutralised by the ammonia derived from the nitrogenous substance under investigation. This 40 c.c. of decinormal acid =4 c.c. of normal acid =4 c.c. of normal ammonia $=4 \times 0{\cdot}017 = 0{\cdot}068$ gramme of ammonia. 0·15 gramme of the substance therefore yields 0·068 gramme of ammonia, and this amount contains 0·056 gramme of nitrogen; 100 grammes of the substance will therefore contain $\frac{100 \times 0{\cdot}056}{0{\cdot}1} = 37{\cdot}3$ grammes of nitrogen.

ANALYSIS OF ORGANIC COMPOUNDS CONTAINING SULPHUR

The method we have described for determining the carbon in sulphur free substances would give too high a result with substances containing sulphur, as the sulphurous acid formed on combustion would be absorbed in the potash bulbs. Carius recommends that the substance containing the sulphur should be burnt with lead chromate in a combustion tube 60–80 cm. long, care being taken that the anterior 10–20 cm. which contains pure lead chromate is never heated above low redness. The pure lead salt retains the sulphurous acid produced by combustion, and thus never contaminates the potash in the bulbs beyond.

Hydrogen and nitrogen are determined by any of the methods described.

As regards the estimation of the sulphur itself, that element is weighed in the form of barium sulphate, into which it may be converted by many methods, the principle of which has been already given under tests for sulphur (1 and 2, pp. 19, 20).

If the substance contains oxygen this is estimated by difference.

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[1] One can guess approximately how much ammonia is expected; in a proteid, for instance, there is speaking roughly 15 per cent. of nitrogen; suppose 0·5 gramme of this is taken, we should obtain in round numbers 0·08 gramme of nitrogen from this, which would be converted into 0·1 gramme of ammonia. 1 c.c. of normal acid corresponds to 0·017 of ammonia; therefore 10 c.c. of normal acid or 100 c.c. of decinormal acid, which is more often employed, would neutralise 0·87 gramme of ammonia, and therefore be quite a safe quantity to take. Decinormal hydrochloric acid is the acid I myself use.

ESTIMATION OF PHOSPHORUS IN ORGANIC SUBSTANCES

The estimation of phosphorus is effected in a manner similar to that of sulphur; *i.e.* the substance is oxidised either in the dry or wet way, and the phosphoric acid produced is determined generally by weighing the precipitate caused by a mixture of magnesium sulphate, ammonium chloride, and ammonia.

Phosphorus cannot be estimated in an organic substance by incinerating and determining the phosphoric acid in the ash, as all the phosphorus is not converted by this means into phosphoric acid.

In analysing animal tissues, one has to deal with substances, such as proteids, to which small quantities of mineral bodies obstinately adhere, and are not removable by any known means before analysis. After analysis one has therefore to incinerate, weigh the ash, and allow for this in subsequent calculations. This method can, however, only be regarded as approximate; first, because certain salts like sodium chloride are volatile to some extent and pass off; and, secondly, because if sulphur or phosphorus, or both (as in nervous tissues), are present, a certain amount of sulphuric or phosphoric acids respectively are formed during ignition; these are weighed with the ash, though they have really been derived from the organic substance itself.

If a substance contains phosphates as well as phosphorus in organic combination, a separate portion is boiled with dilute hydrochloric acid, filtered, and the phosphorus present as phosphoric acid estimated in the solution; this amount is deducted from the total quantity of phosphorus.

DETERMINATION OF IRON IN ORGANIC SUBSTANCES

In some few substances in the body, *e.g.* hæmoglobin, iron is present in addition to the other elements we have mentioned. A weighed amount of the material is incinerated; the ash dissolved in hydrochloric acid, and the amount of ferric chloride so formed ascertained by one of the many volumetric processes now in use. The following is Oudemann's method.[1] To the dilute ferric solution, which should not contain more than 0·1 to 0·2 gramme Fe in 100 c.c., nor much free HCl, 3 c.c. of c. 1 per cent. solution of cupric sulphate are added, 2 c.c. of concentrated HCl, and 1 c.c. of a 1 per cent. solution of potassium sulphocyanide. The mixture is slightly warmed, and a standard solution of sodium thiosulphate (1 c.c. of a decinormal solution of which corresponds to 0·0056 Fe) is run in from a burette, until the previously mixture becomes as perfectly colourless as water.

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[1] *Zeit. Anal. Chem.* vi. 129, and ix. 342.

The amount of iron in hæmoglobin is 0·4 per cent. Knowing this, hæmoglobin may be estimated quantitatively from the amount of iron present in the ash of an unknown amount of the hæmoglobin.

TO DEDUCE EMPIRICAL FORMULÆ FROM PERCENTAGE COMPOSITIONS

From the percentage composition the empirical formula can be calculated, provided that the combining weights of the elements are known. The actual size of the molecule and its constitutional formula are obtained by other methods.

The way in which an empirical formula is deduced may be most readily described by giving examples.

Example 1. Suppose starch has been subjected to elementary analysis and it was found to contain 44·44 carbon, 6·17 hydrogen, and 49·39 oxygen per cent. Knowing that C = 12, H = 1, and O = 16, what is the empirical formula for starch?

Divide the percentage numbers by the combining weights of the elements, and we obtain

$$C_{3·703} \qquad H_{6·17} \qquad O_{3·08}$$

which gives us a rough guide to the formula; from this we can see that the hydrogen atoms are twice as numerous as the oxygen atoms, and the carbon atoms are also rather more numerous than the oxygen atoms. We must next find some common factor which will convert the above numbers into whole numbers; it is, however, generally impossible to do this exactly, and it will be found in the present instance that the number 1·62 is the smallest number which will give us approximately whole numbers, viz.:—

$$C_{5·999} \qquad H_{9·99} \qquad O_{4·97}$$

The nearest whole numbers to these being taken, the simplest empirical formula for starch is $C_6H_{10}O_5$.

Example 2. When we are dealing with a substance containing more than three elements, the arithmetical processes become more complicated. The example I will choose is that of mucin obtained from tendons. Loebisch found that the percentage composition of this material was C, 48·3; H, 6·44; N, 11·75; S, 0·81; O, 32·7. Divide each of these numbers by the combining weight of their respective element, and we obtain a guiding formula, viz.:—

$$C_{4·025} \qquad H_{6·44} \qquad N_{0·83} \qquad S_{0·025} \qquad O_{2·05}$$

It will be found that the lowest common factor which will convert these numbers most approximately into whole numbers is 39 75; this will give us—

$$C_{159·99} \qquad H_{255·99} \qquad N_{32·99} \qquad S_{0·99} \qquad O_{80·48}$$

or approximately, $C_{160}\,H_{256}\,N_{33}\,SO_{80}$. The numbers do not correspond with equal exactness throughout; this is especially noticeable with regard to the oxygen. It must, however, be remembered that there are certain unavoidable small errors of analysis which have always to be allowed for. Moreover, in this particular instance the correspondence between the percentage calculated from the formula and that obtained by analysis is closer than in many other cases.

Such methods give us only an empirical formula; the true molecular weight may be *n* times as great, and in substances in which the molecular weight can be ascertained by determination of vapour density, &c., the calculation is simplified. But with regard to the albuminous and starchy substances we have to deal with in animal chemistry, these methods are not available. The constitutional formula of any substance, *i.e.* the way in which the atoms are united to one another, must be determined by other methods also. Here, again, the substances we have chiefly to deal with in animal chemistry are those, in regard to the constitution of which, we are almost entirely in the dark at present.

CHAPTER IV

GAS ANALYSIS

The gases with which the physiologist has to deal are those of the atmosphere, and those concerned in respiration, those present in the blood and other fluids, as well as those obtainable from the solid tissues of the body. In the greater proportion of cases, a physiologist has to investigate three gases or mixtures of these: viz.:—oxygen, nitrogen, and carbonic acid. Small quantities of carbonic oxide are also produced in the body, and in the alimentary canal, fermentation processes may give rise to hydrogen, marsh gas, and sulphuretted hydrogen. The reader is, however, referred to larger treatises dealing more especially with gas analysis, for the methods of investigating these more rarely occurring gases.

In examining the gases obtainable from either fluid or solid animal tissues, the methods adopted divide themselves naturally into three parts:—

1. The collection of the blood or other tissue in a suitable manner.
2. The extraction of the gases from this material.
3. The analysis of the gases so obtained.

1. METHODS OF COLLECTING MATERIAL INTENDED FOR GAS ANALYSIS

a. Collection of blood. In some cases it is possible to lead the blood direct from the blood-vessel of the animal, by a tube into the vacuum chamber of an air-pump. The gases can be then pumped from it forthwith. The vacuum chamber can be weighed before and after the entrance of blood into it; the increase of weight giving the weight of blood used.

In other cases it is advisable to collect and measure the blood in a separate vessel before introducing it into the air-pump. It must then be collected over mercury, and the following apparatus, as described by Gamgee,[1] answers this purpose admirably. A long graduated tube *ab* is filled with mercury, and placed in connection with a reservoir of mercury *c* by an india-rubber tube; the stopcock at *a* is closed; a narrow elastic tube leading from the blood-vessel is filled with blood, and slipped over the free end *a*, and the stopcock is opened; *b* is also open; the mercury will fall and is replaced by blood. When sufficient blood has been collected, the two stopcocks are closed; the tube is released from the clamp and from the india-rubber tubes at either end, and inverted repeatedly. When blood and mercury are shaken together in this way, fibrin separates from the blood in a very fine state of subdivision. The tube is then laid in a trough containing ice, until the blood within it is wanted for analysis.

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[1] *Physiol. Chem.* p. 181.

b. Collection of other fluids. Here again care must be exercised in obtaining them as fresh as possible, and free from air by collecting them over mercury. The blood-serum, for instance, must be obtained from blood allowed to clot over mercury; the urine, bile, saliva, chyle, &c., are obtained by inserting a cannula into the duct or vessel, as the case may be, and then leading the fluid thence for collection in some such apparatus as that just described for blood.

c. Methods of obtaining solid organs. In the analysis of the gases of a solid organ, such as muscle, a great difficulty is met with at the outset; for the muscles cannot be transferred to the vacuum, without preliminary exposure to air or indifferent fluids. They must be as speedily as possible freed from blood, and plunged instantly into a large volume of boiling salt solution; they will be congulated *en masse*, and die without undergoing the change known as *rigor mortis*. The scalded muscle is at once covered with a beaker filled with the hot

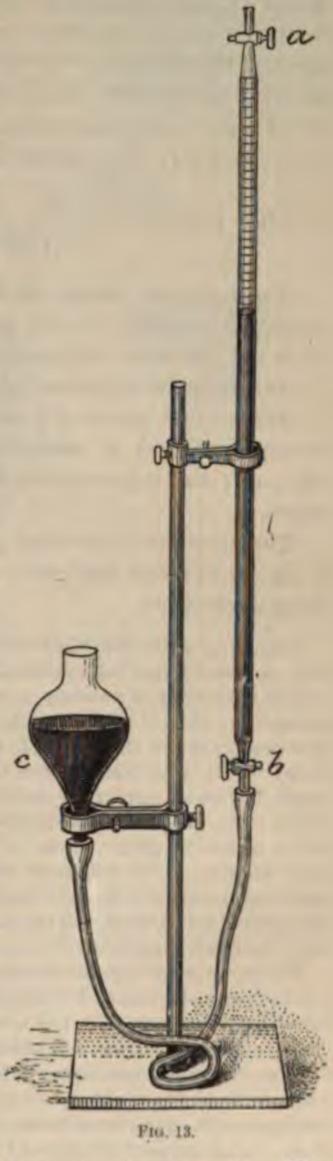

FIG. 13.

saline solution, and any gases that escape are at once collected. The temperature is then lowered, the muscle is minced, and (still contained in salt solution) is introduced into the boiling flask attached to the air-pump. In other cases the muscle is kept from undergoing rigor by being frozen. In other cases, again, the muscle is introduced quickly over mercury into a vessel containing a known volume of air; the changes in the composition of the gases in this closed chamber can be subsequently investigated.

2. THE EXTRACTION OF THE GASES FROM THE MATERIAL UNDER INVESTIGATION

The materials which have been most often investigated are the blood and muscle. It will, therefore, be more convenient to speak of these two, the first an instance of a liquid, the second of a solid tissue.

The gases are extracted by means of a mercurial air-pump.

The earliest forms of pump were made by Ludwig and his pupils, Setschenow[1] and A. Schmidt.[2] The best-known pump is probably Pflüger's; but improvements have been introduced by Alvergniat and others.

The principle of all these pumps may be explained by the diagram in fig. 14, in which the parts of Pflüger's pump are reduced to their simplest elements.

l is a large glass bulb filled with mercury; from its lower end a straight glass tube, *m*, about 3 feet long, extends, which is connected by an india-rubber tube, *n*, with a reservoir of mercury, *o*, which can be raised or lowered as required by a simple mechanical arrangement. From the upper end of the bulb, *l*, a vertical tube passes; above the stopcock, *k*, this has a horizontal branch, which can be closed by the stopcock, *f*. The vertical part is continued into the bent tube, which dips under mercury in the trough, *h*. A stopcock, *j*, is placed on the course of this tube. Beyond *f* the horizontal tube leads into a large double glass bulb, *a b*; a mercurial gauge, *e*, and a drying-tube, *d*, filled with pieces of pumice-stone moistened with sulphuric acid being interposed. *a* is called the blood-bulb, and the blood is brought into it by the tube, *c*; the gases, as they come off, cause the blood to froth, and the bulb, *b*, is called the froth-chamber, as it intercepts the froth, preventing it from passing into the rest of the apparatus.

The pump is used in the following way: *l* is filled with mercury, the level in *l* and *o* being the same; *k* is closed: *o* is then lowered, and when it is 30 inches lower than the stopcock, *k*, the mercury in *l* falls also, leaving that bulb empty: *j* being closed and *f* open, *k* is then opened, and the air in *a*, *b*, *d*, &c., rushes into the Torricellian vacuum in *l*: *f* is closed and *j* opened: the reservoir, *o*, is raised: the mercury in *l* rises also, pushing the air before it, and it bubbles out into the atmosphere through the mercury (the tube, *h*, is not at this stage in position). When *l* is full of mercury, *k* and *j* are once more closed and *o* is again lowered:

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[1] *Zeitsch. f. rat. Med.* 3rd ser. x. 112.

[2] *Ber. d. k. sächs. Gesellsch. d. Wiss.* Leipzig 1867, xix. 33.

when *l* is thus rendered once more a vacuum, *k* and *f* are opened and more of the air remaining in *a*, *b*, *d*, &c., rushes into the vacuum; *f* is closed, *j* is opened, and this air is expelled as before. The process is repeated as often as is necessary to make *a*, *b*, *d*, &c. as complete a vacuum as indicated by the mercury in the gauge, *e*, as is obtainable.

a being now empty and the stopcock, *f*, closed, blood is introduced by the tube, *c*; it froths and gives off all its gases, especially if heated to 40°-45° C. In the case of serum, acid has to be added to disengage the more firmly combined carbonic acid.[1] The bulb, *l*, is once more rendered a vacuum and *k* and *f* are opened, *j* being closed; the gas from *a* and *b* rushes into the bulb, *l*, being dried as it passes through *d*; *f* is then closed and *j* opened; the reservoir, *o*, is raised, and as the mercury in *l* rises simultaneously, it pushes the gases into the

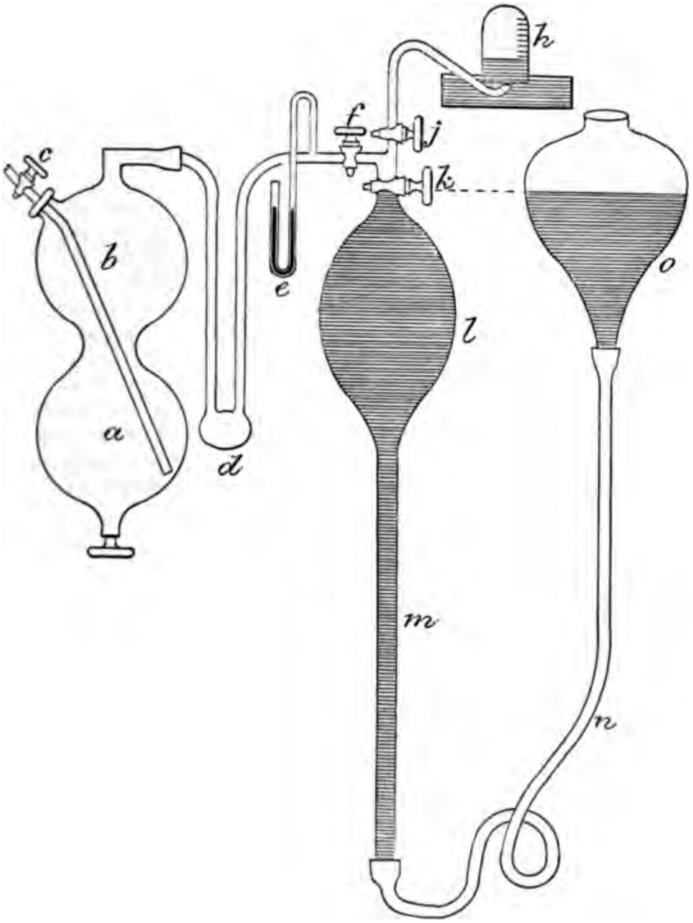

FIG. 14.—Diagram of Pflüger's Pump.

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[1] Phosphoric acid is usually employed.

cylinder, *h*, which is filled with mercury and inverted over the end of the bent tube. This gas can be subsequently analysed. By alternately raising and lowering *o* and regulating the stopcocks in the manner already described, all the gas from the quantity of blood used can be ultimately expelled into *h*.

The large number of stopcocks and joins in Pflüger's pump (for all the parts can be separated) renders leakage apt to occur. A good grease for the taps will be found to be a mixture of two parts vaseline to one of white wax. Alvergniat's[1] pump has the advantage of fewer connections, and all of these are surrounded by mercury, which effectually prevents leakage; it has the disadvantage of a rather small bulb in place of *l*, and thus it is more labour to obtain a vacuum. Dr. McKendrick[2] has also described and figured a small and convenient pump.

In the investigation of the gases of muscle the form of receptacle used is here figured (fig. 15): *a* is a glass bulb into which the muscle contained in boiled salt solution is placed; it is termed the boiling-flask, and is separated from the froth-chamber, *b*, by a tube provided with a stopcock. *a* is perforated by platinum wires which can be attached to a battery and thus stimuli sent into the muscle to cause it to contract if required; *b* serves a second purpose besides that of froth-chamber; a reagent, such as an acid, may be kept in it during the preliminary exhaustion of the muscle in *a*, and then, by tilting the apparatus, may be brought to play on the muscle at any given moment.

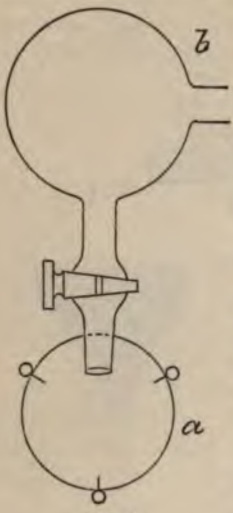

FIG. 15.

3. ANALYSIS OF THE GASES

For gas analysis an accurately graduated and calibrated tube which is closed at the upper end, and then inverted over mercury, is used. It is called a eudiometer, and it has, passing through the glass, two platinum wires which can be connected with an induction coil, and thus powerful sparks can be produced in the interior of the tube in cases where explosion of gases is necessary.

Some gases may be estimated directly, that is, they may be absorbed by certain reagents, the diminution in volume indicating the quantity of gas present. Some are determined indirectly, that is, exploding them with other gases, and measuring the quantities of the products. The gases which are estimated directly are (1) those like hydrochloric acid which are absorbed either by crystallised sodic phosphate or potassium hydrate; (2) those like carbonic acid and sulphurous acid which are absorbed by potassium hydrate and not by sodic phosphate; and (3) those which are absorbed by neither of these two reagents, but by others specially adapted to meet the case in question; in this class of gases are oxygen, carbonic oxide, nitric oxide, and many gaseous hydrocarbons with the exception of marsh gas. The gases estimated indirectly are hydrogen, nitrogen, marsh gas, carbonic oxide, and several others. As an instance of this last class, hydrogen may be selected; a known volume of oxygen is mixed with it in the eudiometer, and exploded; water is formed which condenses, the remaining oxygen is estimated directly; the loss of oxygen after explosion must be that quantity which has combined with hydrogen; and knowing the proportion of hydrogen and oxygen in water, the hydrogen can be thus ascertained.

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[1] Bert, *Leçons sur la respiration*, Paris, 1870.

[2] *Brit. Med. Journ.* Aug. 18, 1888.

As has been stated already, the physiologist has to deal chiefly with three gases: oxygen, carbonic acid, and nitrogen. The first two can be estimated directly by absorption, the residue is nitrogen.

The tube containing the gases is made to stand in a well-shaped pneumatic trough filled with mercury, and by the aid of a pipette turned up at the end, about half a cubic centimetre of strong solution of caustic potash (sp. gr. 1·2) is introduced; when absorption is complete, and this can be hastened by alternately raising and lowering the tube in the mercury, the loss of volume gives the quantity of carbonic acid previously present. About half a c.c. of strong pyrogallic acid (1 of acid to 8 of water) is then introduced; by this reagent the oxygen is absorbed; and the residue is nitrogen.

Greater accuracy, however, is obtained by using solid instead of liquid reagents. The solid is made into the form of a bullet, which can be introduced by a platinum wire through the mercury into the mixture of gases, and withdrawn when no further diminution of volume takes place. Thus, bullets of caustic potash are used for absorbing carbonic acid; for oxygen, phosphorus was formerly used, but now *papier-mâché* balls moistened with a freshly prepared alkaline solution of potassium pyrogallate are more generally employed. As a rule these solid substances must be left some hours in the gas before absorption is complete.

Using liquid reagents I have found that the principal tube of Lunge's nitrometer gives very accurate results, and the way I have been accustomed to use it is as follows:

The apparatus consists of an accurately graduated tube *a*, which tapers at its upper end, and then again widens into the tube *b*; in this narrow neck is a stop-cock, which is perforated by holes in such a way that in one position (*see* A) *a* and *b* are put into communication with one another; and in another position (*see* B) *b* is put into communication with the air, or with any other vessel by means of an india-rubber tube *d*.

a is filled up to the stop-cock with mercury, and placed in a trough containing mercury. The whole apparatus can be raised and lowered by a rack and pinion arrangement, such as one uses to raise and lower the tube of a microscope. The stop-cock *c* is placed intermediate in position between A and B, so that *a* is in communication neither with *b* nor *d*. The gas to be analysed is then introduced into the tube *a* in one of two ways : either through the mercury at the lower opening of the tube ; or by placing the interior of *a* into communication with a vessel containing the gases, by means of a tightly fitting india-rubber tube *d*, the stop-cock being placed in the position B ; at a given moment, a clip is removed from the tube *d*, and the gases pass into *a*, the mercury falling in that tube until it has the same level outside and inside. The clip is then replaced, the stop-cock once more put in a neutral position, and the volume of gas read off. The whole tube is now raised so that the mercury within it stands at a higher level than that outside. About half a c.c. of strong potash solution is put into the tube *b*, the stop-cock turned into the position A, and the potash trickles through into the tube *a* ; this absorbs the carbonic acid, the apparatus is then adjusted so that the mercury within and without *a* is at the same level ; and the remaining gases read off. The oxygen is then absorbed by running in pyrogallic acid solution in the same way, and the residual nitrogen read off over mercury, or better over water ; in the latter case, the gas is read off in the moist state and the water within and without the tube *a* must be at the same level.

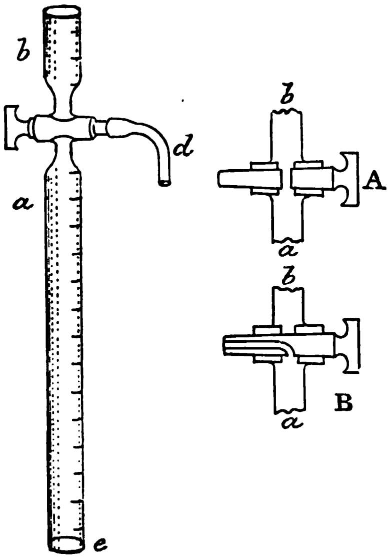

FIG. 16.

In order to get results which are comparable with one another, the alteration in the volume of gases produced by temperature, barometric pressure, and if moist, by tension of aqueous vapour must be always allowed for, and the volume corrected to standard pressure (760 mm.) of mercury, and standard temperature (0° C.). The following formula serves for correcting volumes of gases :

V^1 = the correct volume.
V = the volume observed.
B = height of barometer (which should in very accurate work be also corrected for temperature).
t = temperature in degrees centigrade.
T = tension of aqueous vapour in millimetres of mercury at $t°$ (*see* table, p. 5).

Then,
$$V^1 = \frac{V \times (B - T)}{760 \times (1 + 0{\cdot}003665t)}$$

If the gas is dry, then
$$V^1 = \frac{V \times B}{760 \times (1 + 0{\cdot}003665t)}$$

The number 0·003665 is the coefficient of expansion of gases.

The number $760 \times (1 + 0{\cdot}003665t)$ is obtained from tables, and the calculations are much simplified by the use of logarithms: thus,

$$\log V^1 = \log V + \log (B - T) - \log [760 \times (1 + 0{\cdot}003665t)],$$

or, for dry gases,

$$\log V^1 = \log V + \log B - \log [760 \times (1 + 0{\cdot}003665t)].^1$$

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[1] Mr. F. Sutton, Norwich, will forward a copy of these tables, printed separately for laboratory use, to any one desiring them, on receipt of the necessary address.

CHAPTER V

OPTICAL INSTRUMENTS USED IN CHEMICO-PHYSIOLOGICAL INVESTIGATIONS

THE MICROSCOPE

This instrument is of value in observations on crystals which are too small to be seen, much less measured, by the naked eye. In performing chemical reactions with small quantities of material, it is sometimes convenient to do so on a microscope slide, and observe the result with the microscope. Such operations are designated micro-chemical. A familiar instance of a micro-chemical reaction is the test for blood, which consists in the formation of hæmin crystals.

POLARISATION OF LIGHT

If an object, such as a black dot on a piece of white paper, be looked at through a crystal of Iceland spar, two black dots will be seen ; and if the crystal be rotated, one black dot will move round the other, which remains stationary. That is, rays of light entering such a crystal are split into two rays, which travel through the crystal with different velocities, and consequently one is more refracted than the other. One ray travels just as it would through glass ; this is the *ordinary ray*, the ray which gives the stationary image ; the other ray gives the movable image when the crystal is rotated ; the ordinary laws of refraction do not apply to it, and it is called the *extraordinary ray*. Both rays are of equal brilliancy. In one direction, however, that of the optic axis of the crystal, a ray of light is transmitted without double refraction.

Ordinary light, according to the wave theory, is due to vibrations occurring in all planes transversely to the direction of the propagation of the wave. Light is said to be plane polarised when the vibrations take place all in one plane. The two rays produced by double refraction are both polarised, one in one plane, the other in a plane at right angles to this one. Doubly refracting bodies are called *anisotropous* ; singly refracting bodies, *isotropous*. The effect of polarisation may be very roughly illustrated by a model.

If a string be stretched as in the figure, and then touched with the finger, it can be made to vibrate, and the vibrations will be free to occur from above down, or from side to side, or in any intermediate position. If, however, a disc with a vertical slit be placed on the course of the string, the vibrations will be all obliged to take place in a vertical plane, any side to side movement being stopped by the edges of the slit.[1]

Light can be polarised not only by the action of crystals, but by reflection from a surface at an angle which varies for different substances (glass 54° 35′, water 52° 45′, diamond 68°, quartz 57° 32′, &c.). It is also found that certain non-crystalline substances, like muscle, cilia, &c., are doubly refracting.

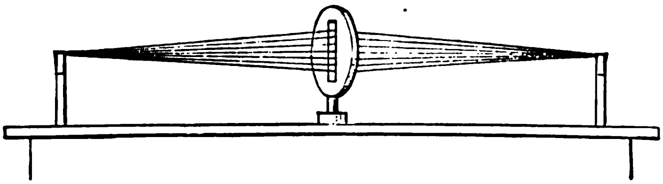

Fig. 17.

The Nicol's Prism is the *polariser* usually employed in polariscopes; it consists of a rhombohedron of Iceland spar divided into two by a section through its obtuse angles. The cut surfaces are polished and cemented together in their former position with canada balsam. By this means the ordinary ray is totally reflected through the canada balsam ; the extraordinary ray passes on and emerges in a direction parallel to the entering ray. In this polarised ray there is nothing to render its peculiar condition visible to the naked eye ; but if the eye is aided by a second nicol's prism, which is called the *analyser*, it is possible to detect the fact that it is polarised.

This may be again illustrated by reference to our model (fig. 18).

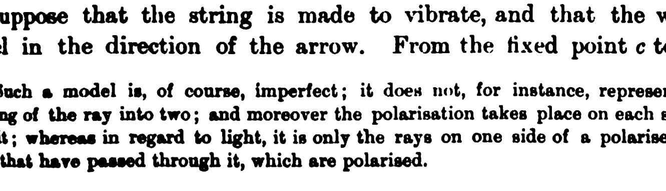

Fig. 18.

Suppose that the string is made to vibrate, and that the waves travel in the direction of the arrow. From the fixed point *c* to the disc *a*, the string is theoretically free to vibrate in any plane ;[1] but after passing through the vertical slit in *a*, the vibrations must all be vertical also : if a second similar disc *b* be placed further on, the vibrations will also pass on freely to the other extremity of the string *d*, if as in the figure (fig. 18) the slit in *b* be also placed vertically. If, however, *b* be so placed that its slit is horizontal (fig. 19), the vibrations will be extinguished on reaching *b*, and the string between *b* and *d* will be motionless.

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[1] Such a model is, of course, imperfect; it does not, for instance, represent the splitting of the ray into two; and moreover the polarisation takes place on each side of the slit; whereas in regard to light, it is only the rays on one side of a polariser, viz. those that have passed through it, which are polarised.

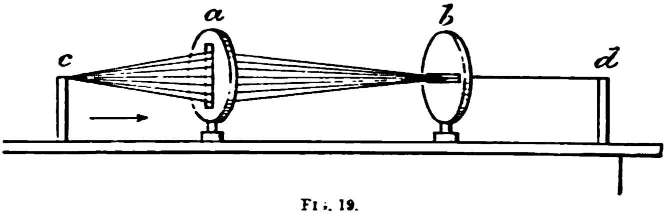

FIG. 19.

c here represents a source of light, and the vibrations of the string the undulations which by the nicol's prism *a* are polarised so as to occur in one plane only ; if the second nicol or the analyser *b* is parallel to the first, the vibrations will pass on to the eye, which is represented by *d* ; but if the planes of the two nicols are at right angles, the vibrations allowed to pass through the first are extinguished by the second, and so no light reaches the eye. In intermediate positions, *b* will allow only some of the light to pass through it. It must be clearly understood that a nicol's prism contains no actual slits, but the arrangement of its molecules is such, that their action on the particles of æther may be compared to the action of slits in a diaphragm to vibrations of more tangible materials than æther.

The Polarising Microscope consists of an ordinary microscope with certain additions : below the stage is the polarising nicol : in the eye-piece is the analysing nicol ; the eye-piece is so arranged that it can be rotated ; thus the directions of the two nicols can be made parallel and then the field is bright ; or crossed, and then the field is dark. The stage of the microscope is arranged, so that it also can be rotated.

The polarising microscope is used to detect doubly refracting substances. Let the two nicols be crossed, so that the field is dark ; interpose between the two, that is, place upon the stage of the microscope, a doubly refracting plate of which the principal plane is parallel to the first prism or polariser : the ray from the first prism is unaffected by the plate, but will be extinguished by the second ; the field therefore still remains dark. If the plate is parallel to the second nicol the field is also dark; but in any intermediate position, the light will be transmitted by the second nicol. In other words, if between two crossed nicols, which consequently appear dark, a substance be interposed which in certain positions causes the darkness to give place to illumination, that substance is doubly refractive. How this takes place may be explained as follows :—

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[1] The imperfection of the model has been already explained.

Let N_1N_1 (fig. 20) represent the direction of the principal plane of the first nicol, and N_2N_2 that of the second. They are at right angles, and so the ray transmitted by the first, will be extinguished by the second. Let PP represent the principal plane of the interposed doubly refractive plate. The extraordinary ray transmitted by N_1N_1 vibrates in the plane N_1N_1, and falls obliquely on the plate PP ; it is by this plate itself split into two rays, an ordinary and an extraordinary one, at right angles to one another, one vibrating in the plane PP, the other in the plane P^1P^1. These two rays meet the second nicol, which can only transmit vibrations in the plane N_2N_2. The vibrations in PP can be resolved into a vibration in N_1N_1 and a vibration in N_2N_2, the former is extinguished, the latter transmitted. Similarly the vibration in P^1P^1 can be resolved into two sub-rays in N_1N_1 and N_2N_2 respectively, the latter only being transmitted. The illumination is thus due to two sub-rays, one of the vibrations in PP, the other of those in P^1P^1 which have been made to vibrate in N_2N_2.

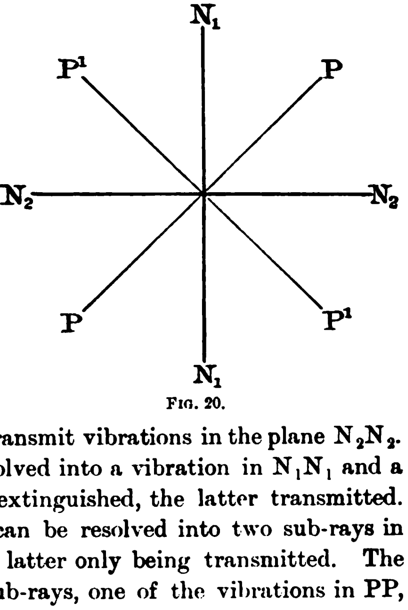

FIG. 20.

Now, although these two sub-rays vibrate in the same plane, they are of different velocities ; hence the phases of the vibrations do not coincide, and thus the phenomena of interference are obtained. If we have two sets of vibrations fused, the crest of one wave may coincide with the crest of the other, in this case the wave will be higher ; or the crest of one may coincide with the hollow of the other; that is, the undulation would be extinguished ; in other intermediate cases, the movement would be interfered with, either helped or hindered more or less. Interference in the case of many kinds of doubly refracting substances (Iceland spar is in this an exception) shows itself in the extinction of certain rays of the white light, and the light seen through the second nicol is white light *minus* the extinguished rays ;

those extinguished and those transmitted will together form white light, and are thus complementary. Moreover, the rays extinguished in one position of the plate will be transmitted in one at right angles and *vice versa*; thus a crystal showing these phenomena of *pleochromatism* as it is termed, will transmit one colour in one position, and the complementary colour in a position at right angles to the first; blue and yellow, and red and green, are the pairs of colours most frequently seen in this way.

The subject of double refraction and polarisation of light will be discussed in certain special aspects in connection with hæmoglobin crystals and muscle.

Rotation of the plane of Polarisation.—Certain crystals such as those of quartz, and certain fluids such as the essence of turpentine, aniseed, &c., and solutions of certain substances like sugar, and albumin, have the power of rotating the plane of polarised light to the right or left. The polarisation of light that is produced by a quartz crystal is different from that produced by a rhombohedron of Iceland spar. The light that passes through the latter is plane polarised; the light that passes through the former (quartz) is circularly polarised; i.e. the two sub-rays are made up of vibrations which occur not in a plane, but are curved. The two rays are circularly polarised in opposite directions, one describing circles to the left, the other to the right; these unite on issuing from the quartz plate; and the net result is a plane polarised ray with the plane rotated to right or left according as the right circularly polarised ray or the left proceeded through the quartz with the greater velocity. There are two kinds of quartz, one which rotates the plane to the right (dextrorotatory), the other to the left (lævorotatory).

Gordon[1] explains this by the following mechanical illustration. Ordinary light may be represented by a wheel travelling in the direction of its axle, and the vibrations composing it executed along any or all of its spokes (*a*). If the vibrations all take place in the

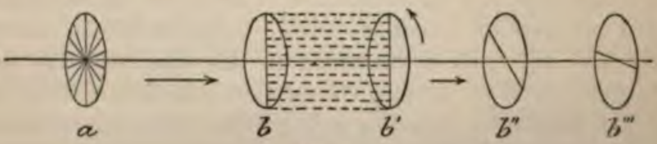

FIG. 21.

same direction, i.e. along one spoke, and the spoke opposite to it (*b*), the light is said to be plane polarised. The two spokes as they travel along in the direction of the arrow will trace out a plane (*see* figure 21) between *b* and *b*′. If this polarised beam be made to travel now through a solution of sugar, the net result is that the plane so traced out is twisted or rotated; the two spokes, as in *bb*′, do not trace out a plane, but we must consider that they rotate as they travel along, as though guided by a spiral or screw thread cut on the axis, so that after a certain distance the vibrations take place as in *b*″; later in *b*‴, and so on. This effect on polarised light is due to the molecules in solution, and the amount of rotation will depend on the strength of the solution, and on the length of the column of the solution through which the light passes; or in the case of a quartz plate on its thickness.

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[1] *Physical Treatise on Electricity and Magnetism.*

If a plate of quartz be interposed between two nicols, the light will not be extinguished in any position of the prisms, but will pass through various colours as rotation is continued. The rotation produced for different kinds of light being different, white light is split into its various constituent colours; and the angle of rotation that causes each colour to disappear is constant for a given thickness of quartz plate, being least for the red and greatest for the violet. These facts are made use of in the construction of polarimeters. Polarimeters are instruments for determining the strength of solutions of sugar, albumin, &c., by the direction and amount of rotation they produce on the plane of polarised light. They are often called saccharimeters, as they are specially useful in the estimation of sugar.

Soleil's Saccharimeter.—This instrument (*see* fig. 22) consists of a nicol's prism *d*, called the polariser; the polarised ray passes next through a quartz plate (*b*) 3·75 m.m. thick, one half (*d* in fig. 23) of which is made of dextrorotatory, the other half (*g* in fig. 23) of lævorotatory quartz.

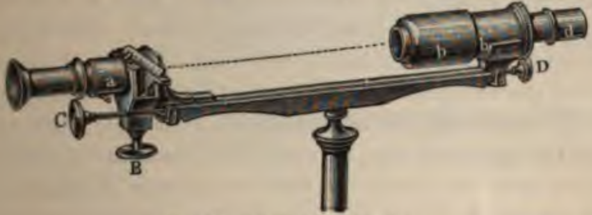


FIG. 22.—Soleil's Saccharimeter.

The light then passes through the tube containing the solution placed in the position of the dotted line in fig. 22, then through a quartz plate cut perpendicularly to its axis (*g* in fig. 23), then through an arrangement called a compensator (*r* in fig. 23), then through a second nicol (*a*) called the analyser, and lastly through a telescope (L in fig. 23).

The compensator consists of two quartz prisms (RR, fig. 23) cut perpendicularly to the axis, but of contrary rotation to the plate just in front of them. These are wedge-shaped and slide over one another, the sharp end of one being over the blunt end of the other. By a screw the wedges may be moved from one another, and this diminishes the thickness of quartz interposed; if moved towards one another the amount of quartz interposed is increased.

The effect of the quartz plate (*d*, *g*) next to the polariser (*c* in fig. 23) is to give the polarised light a violet tint when the two nicols are parallel to one another. But if the nicols are not parallel, or if the plane of the polarised light has been rotated by a solution in the tube, one half the field will change in colour to the red end, the other to the violet end of the spectrum, because the two halves of the quartz act in the opposite way.

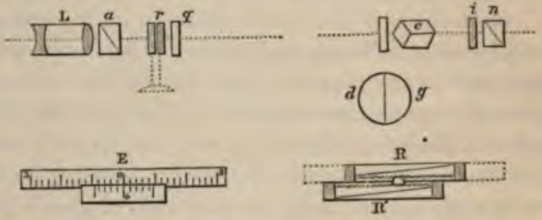


FIG. 23.—Diagram of optical arrangements in Soleil's Saccharimeter.

The instrument is first adjusted with the compensator at zero, and the nicols parallel, so that the whole field is of one colour. The tube containing the solution is then interposed; and if the solution is optically inactive the field is still uniformly violet. But if the solution is dextrorotatory the two halves will have different tints, a certain thickness of the compensating quartz plate which is lævorotatory must be interposed to make the tint of two halves of the field equal again; the thickness so interposed can be read off in amounts corresponding to degrees of a circle by means of a vernier and scale (E in fig. 23) worked by the screw which moves the compensator. If the solution is lævorotatory, the screw must be turned in the opposite direction.

Zeiss's Polarimeter is in principle much the same as Soleil's; the chief difference is that the rotation produced by the solution is corrected not by a quartz compensator, but by actually rotating the analyser in the same direction, the amount of rotation being directly read off in degrees of a circle.

Laurent's Polarimeter is a more valuable instrument. Instead of using daylight, or the light of a lamp, monochromatic light (generally the sodium flame produced by volatilising common salt in a colourless gas flame) is employed; the amount of rotation varies for different colours; and now all observations are recorded as having been taken with light corresponding to the D or sodium line of the spectrum. The essentials of the instrument are as before, a polariser, a tube for the solution, and an analyser. The polarised light before passing into the solution traverses a quartz plate, which however covers only half the field, and retards the part of the ray passing through it by half a wave-length. In the 0° position the two halves of the field appear equally illuminated; in any other position, or if rotation has been produced by the solution when the nicols have been set at zero, the two halves appear unequally illuminated. This is corrected by means of a rotation of the analyser, that can be measured in degrees by a scale attached to it.

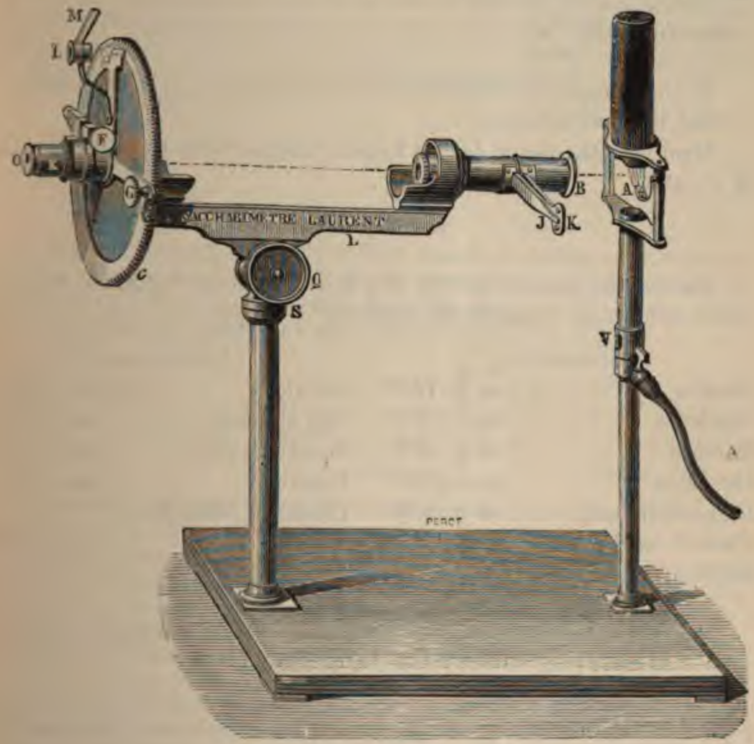


FIG. 24.—Laurent's Polarimeter.

Specific Rotatory power of any substance is the amount of rotation in degrees of a circle of the plane of polarised light produced by

1 gramme of the substance dissolved in 1 c c. of liquid examined in a column 1 decimetre long.

If a = rotation observed.
w = weight in grammes of the substance per cubic centimetre.
l = length of the tube in decimetres.
$(a)_D$ = specific rotation for light with wave-length corresponding to the D line (sodium flame)

then $(a)^D = \pm \frac{a}{wl}$ [1].

In this formula + indicates that the substance is dextrorotatory; − that it is lævorotatory.

If on the other hand $(a)_D$ is known, and we wish to find the value of w; then

$$w = \pm \frac{a}{(a)_D \times l}$$ [1]

The specific rotatory powers of a few of the more important optically active substances found in the body are as follows:—

Dextrorotatory		*Lævorotatory*	
Sucrose . .	$(a)_D = +\ 73\cdot8°$	Levulose . .	$(a)_D = -106°$
Dextrose . .	$= +\ 56\cdot0°$	Egg albumin. . .	$= -\ 33\cdot5°$
Lactose . . .	$= +\ 59\cdot3°$	Serum-albumin . .	$= -\ 56\cdot0°$
Dextrine. . .	$= +138\cdot8°$	Gelatin . . .	$= -130°$
Glycocholic acid .	$= +\ 29\cdot0°$	Chondrin (alkaline solution). . .	$= -213\cdot5°$
Cholalic acid . .	$= +\ 35\cdot0°$		
Sodium taurocholate	$= +\ 24\cdot5°$		

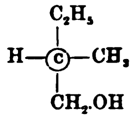
RELATION BETWEEN CIRCULAR POLARISATION AND CHEMICAL CONSTITUTION

The first work in this direction was performed by Pasteur,[2] and it was his publications on this subject that brought him into prominence. He found that racemic acid, which is optically inactive, can be decomposed into two isomerides, one of which is common tartaric acid which is dextrorotatory, and the other tartaric acid differing from the common variety in being lævorotatory. The salts of tartaric acid usually exhibit hemihedral faces, while those of racemic acid are holohedral. Pasteur found that, although all the tartrate crystals were hemihedral, the hemihedral faces were situated on some crystals to the right, and on others to the left hand of the observer, so that one formed, as it were, the reflected image of the other. These crystals were separated, purified by recrystallisation, and those which exhibited dextro-hemihedry possessed dextrorotatory power, whilst the others were lævorotatory. Pasteur[1] further showed that if the mould ***penicillium glaucum*** be grown in a solution of racemic acid, dextro-tartaric acid first disappears, and the lævo-acid alone remains. The subject remained in this condition for many years; it was, however, conjectured that probably there is some molecular condition corresponding to the naked eye crystalline appearances which produces the opposite optical effects of various substances. What this molecular structure was, was pointed out independently by two observers—Le Bel[2] in Paris, and Van 't Hoff[3] in Holland—who published their researches within a few days of each other. They pointed out that all optically active bodies contain one or more assymmetric carbon atoms, i.e. one or more atoms of carbon connected with four dissimilar groups of atoms, as in the following examples:—

---

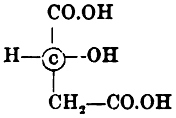
[1] As solution may cause condensation of volume, the density (d) or specific gravity of the solution should be also taken; then $(a)_D = \pm \frac{a}{w\,l\,d}$ and $w = \pm \frac{a}{(a)_D \times ld}$.

[2] *Ann. Chim. Phys.* (2) xxiv. 442; xxviii. 56. *Comptes rend.* xxxvi. 26; xxxvii. 162. *Poggendorff's Annalen*, lxxx. 127; xc. 498, 504.



$$\begin{array}{c} C_2H_5 \\ | \\ H-\text{\textcircled{c}}-CH_3 \\ | \\ CH_2.OH \end{array}$$

Amyl alcohol



$$\begin{array}{c} CO.OH \\ | \\ H-\text{\textcircled{c}}-OH \\ | \\ CH_2-CO.OH \end{array}$$

Malic acid

The question, however, remained—do all substances containing such atoms rotate the plane of polarised light? It was found that they do not; this is explained by Le Bel by supposing that these, like racemic acid, are compounds of two molecules—one dextro-, the other lævo-rotatory; that this was the case was demonstrated by growing moulds, the fermenting action of which is to separate the two molecules in question. Then the other question—how is it that two isomerides, which in chemical characteristics, in graphic as well as empirical formula, are precisely alike, differ in optical properties?—is explained ingeniously by Van 't Hoff. He compares the carbon atom to a tetrahedron with its four dissimilar groups, A, B, C, D, at the four corners. The two tetrahedra represented in fig. 25 appear at first sight precisely alike; but if one be super-imposed on the other, C in one and D in the other could never be made to coincide. This difference cannot be represented in any other graphic manner, and probably represents the difference in the way the atoms are grouped in the molecule of right- and left-handed substances respectively.

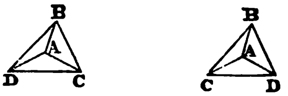


FIG. 25.

THE SPECTROSCOPE

When a ray of light passes from one medium such as air into another such as water or glass, it is bent out of its original course, or as it is termed refracted. The ratio of the sine of the angle of incidence to that of the angle of refraction is called the refractive index. When white light is passed through a glass prism or a triangular bottle containing a substance like carbon bisulphide with a high refractive index, it undergoes two bendings, one at each surface of the prism; the whole ray is however not equally bent, but it is split into its constituent colours, which may be allowed to fall on a screen; the red rays will be found at one end of a continuous band of colour, the violet rays at the other; orange, yellow, green, blue and indigo being the colours which are intermediate in the order named. This band of colours is termed a *spectrum*. The rainbow is an instance of a spectrum produced in nature by the sun's rays passing at a certain angle through drops of water. A spectroscope is an instrument provided with a prism or prisms to enable us to obtain a spectrum artificially. In the spectrum the red rays are the least, the violet rays the most, refrangible. If the spectrum produced by one prism be immediately passed through a second prism like the first, but inverted, the coloured rays are reunited, and build up a white ray emerging from the second prism.

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[1] *Compt. rend.* li. 158.

[2] *Bull. Soc. Chim.* (2) xxii. 337.

[3] *La chimie dans l'espace.*

The different colours are due to vibrations of æther of different rates of rapidity; the wave-length of red light is greater than that of yellow, that of yellow than that of green, and so on, violet having the shortest wave-length. The wave-length of the ray changes in different media, and thus the velocity of propagation varies; the violet is the most, the red the least, retarded; the violet is thus bent the most, the red the least; hence, arises the dispersion which results in the formation of the spectrum.

In addition to the visible rays, other rays at either end are present which can be detected by their effects, though not by the eye. These rays are called respectively the ultra-red and the ultra-violet. The ultra-red rays are those in which heating effects preponderate, the ultra-violet are rays of chemical activity, i.e. produce such chemical changes as those on which the art of photography depends; the visible rays have heating, and chemical effects also, but in a subordinate degree.

The spectrum of sunlight is interrupted by numerous dark lines crossing it vertically, called Fraunhofer's lines. They are perfectly constant in position, and serve as landmarks in the spectrum: the more prominent are lettered; A, B, and C are in the red, D in the yellow, E and F in the green, G in the indigo, and H in the violet. The *a* (in the red) and *b* lines (in the green) are also well marked. These lines are due to the presence of certain substances volatilised in the solar atmosphere. If the light from burning sodium or its compounds be examined spectroscopically, it will be found to give a bright yellow line, or rather two bright yellow lines, very close together; it is in fact a true monochromatic light. Potassium gives two bright red lines, and one violet line, and the other elements when incandescent give characteristic lines, but none so simple as sodium. If now the flame of a lamp be examined, it will be found to give a continuous spectrum, like the solar spectrum in the arrangement of its colours, but unlike it in the absence of dark lines; but if the light from the lamp be made to pass through sodium vapour (produced by burning salt in an ordinary spirit flame) before it reaches the spectroscope, the bright yellow light will be found absent, and in its place a dark line, or rather two dark lines close together, occupying the same position as the two bright lines of the sodium spectrum. The sodium vapour thus absorbs the same rays as those which it itself produces at a higher temperature. Thus the D line as we term it in the solar spectrum is due to the presence of sodium vapour in the solar atmosphere. The other dark lines are also all due to the absence of certain rays, absorbed by the presence of such substances as hydrogen, calcium, barium, iron, &c., in a volatile condition in the sun's atmosphere; it being a general rule that the vapour of any material will absorb and retain light, the period of vibration of which is identical with that which it itself emits when in a state of incandescence.

The Spectroscope consists of a tube A called the collimator, with a slit at the end S, and a convex lens at the end L; the latter makes the rays of light passing through the slit from the source of light parallel: they fall on the prism, are refracted, and then the spectrum so formed is focussed by the telescope T. The dispersion of the colours, and so the length of the spectrum, may be increased by using a train of prisms in place of P, the second prism being so placed as to receive the rays refracted from the first, the third those from the second, and so on. There are in addition various accessories to the instrument: e.g. most spectroscopes have a third tube which carries a small transparent scale

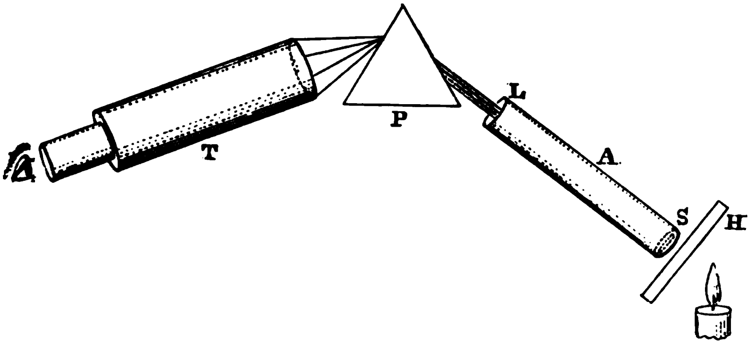

FIG. 26.

of wave-lengths; this is illuminated and is focussed by the telescope. Generally also a small rectangular prism is placed in front of the lower part of the slit at S; the solar light is focussed on to this, and we thus have two spectra, one of the candle flame or of the substance under examination below, and the solar spectrum above which can be compared with it, and lastly an image of the scale by which the position of any line or band can be read off in wave-lengths.

If we now interpose between the source of light and the slit S a piece of coloured glass H, or a solution of a coloured substance contained in a vessel with parallel sides (the hæmatoscope of Hermann, fig. 27, F), the spectrum will be found to be no longer continuous, but interrupted by a number of dark bands corresponding to the light absorbed by the coloured medium. Thus oxyhæmoglobin gives two perfectly characteristic bands between the D and E lines, hæmoglobin giving only one; and other red solutions, though to the naked eye similar to oxyhæmoglobin, will give characteristic bands in other positions. Chlorophyll again gives four well marked bands, especially one in the red. The study of the absorption spectra of animal and vegetable pigments is full of interest, and has been followed with most valuable results (*see* further chlorophyll, hæmoglobin, bile, urine, &c.).

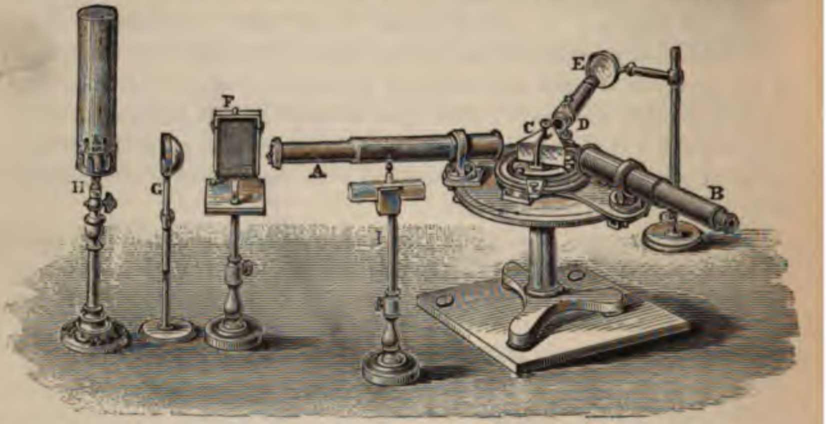

FIG. 27.—Spectroscope. A, collimator with adjustable slit at one (left) end and collimating lens at the other (right) end; B, telescope moving on graduated arc divided into degrees; C, prism or combination of prisms; D, tube for scale; E, mirror for illuminating scale; F, vessel with parallel glass sides for holding fluid, shown with the flat side towards the reader; I, long spectroscope bottle for examining a deep layer of fluid; H, Argand burner; G, condenser for concentrating the light from H on the slit. (From a photograph taken by Dr. MacMunn, from McKendrick's 'Physiology.')

A convenient form of small spectroscope is the *direct vision spectroscope*, in which by an arrangement of alternating prisms of crown and flint glass, placed as in fig. 28, the spectrum is observed by the eye in the same line as the tube furnished with the slit; indeed slit and prisms are both contained in one tube.

The Microspectroscope is a spectroscope fitted into the ocular end of a microscope, instead of the eye-piece. There are slight variations in the instruments constructed by Browning, Hilger, and Zeiss. The light which passes up through the microscope tube, passes through a direct vision spectroscope; the spectrum so formed is compared with a spectrum from solar light which enters by a slit in the side of the instrument, and is then by a small rectangular prism sent up also through the tube of the microspectroscope; and lastly an illuminated scale is focussed with these, as in the ordinary spectroscope.

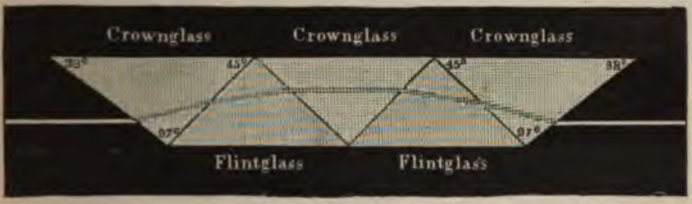

FIG. 28.—Arrangement of Prisms in direct vision Spectroscope.

The microspectroscope is of value in examining spectroscopically small quantities of solutions; small cells for containing the fluid to be examined are made from short pieces of barometer tubing cemented to microscope slides. In examining aqueous extracts of blood stains on garments, very often only a small volume of liquid can be obtained; in order to identify the blood pigment spectroscopically, one must here have recourse to the microspectroscope.

The instrument is also useful in examining coloured microscopic crystals, or coloured portions of microscopic organisms. Dr. MacMunn has made much use of the microspectroscope in this direction. He adopts the following method: a binocular microscope is taken; the microspectroscope is put in the place of one eye-piece. By means of the other eye-piece, the portion of tissue or crystal can be accurately focussed; its absorption spectrum is then seen on looking down the spectroscope in the other tube.

The absorption bands which form the characteristic features of blood and other animal liquids do not admit of having their limits determined with the same precision as is possible in the case of Fraunhofer's lines; their position in wave-lengths is usually determined in millionths of a millimetre, instead of ten-millionths. The edges of absorption bands are moreover sometimes so ill-defined, and vary so

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much with the concentration of the solution, that often their centre is given, instead of the position of their edges.

Printed blank maps (similar to fig. 29) accompany some of Zeiss's instruments, and correspond exactly to the scale of the spectroscope. It is thus easy to draw a diagram of any given spectrum. The observer commences by causing the D or sodium line to coincide exactly with that part of the scale which expresses its wave-length, that is to say, with the division 589 of the scale (this expresses the fact that the wave-length usually denoted by the Greek letter λ is 589 millionths of a millimetre). Having done this, the scale is set accurately for all other points.

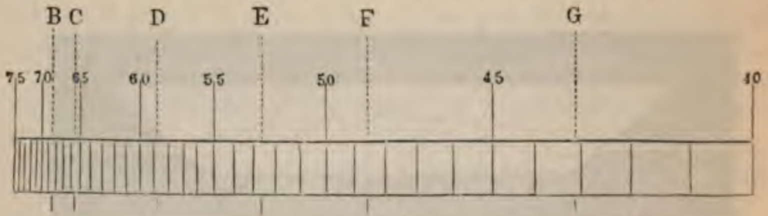

FIG. 29.—Scale of Wave-lengths.

The usual method of determining wave-lengths, namely by interpolation curves, is thus described by MacMunn :—[1]

> A piece of paper ruled into square inches and tenths, obtainable from Letts & Co., has a scale of wave-lengths ruled off along the right-hand edge, and the upper edge at right angles to this has a scale corresponding to the scale of the instrument marked on it. The value of the Fraunhofer lines on the scale of the spectroscope is observed, and by a reference to Angström's numbers, their value in wave-lengths;[2] they are then marked in their proper places on the scale with dots. A curve is then drawn through these marks as uniformly as possible. When a band or bright line has to be mapped out, all that is necessary is to take its reading on the scale; then knowing between what lines it is placed, we find its position on the curve opposite which its wave-length is printed on the right-hand edge.

THE SPECTROPHOTOMETER

The spectrophotometric method for estimating the concentration of coloured solutions was originally proposed by Bunsen and Roscoe,[3] in 1857. In 1873 Vierordt[4] invented a spectrophotometer, but it is Hüfner[5] who definitely introduced the instrument into physiological methods.

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[1] *The Spectroscope in Medicine*, p. 32.

[2] Angström's calculations of the wave-lengths of the principal Fraunhofer lines are as follows in millionths of a millimetre: A, 760·4; *a*, 718·5; B, 686·7; C, 656·2; D, 589·2; E, 526·9; *b*, 517·2; F, 486·0; G, 430·7; H_1, 396·8; H_2, 393·3. (*Recherches sur le spectre solaire.* Upsala, 1865.)

[3] Poggendorff's *Annalen*, vol. ci. p. 235.

[4] Vierordt, *Die Anwendung des Spectralapparates zur Photometrie der Absorptionsspectrum und zur quant. chem. Analyse*, Tübingen 1873, and

[5] Hüfner, *Journ. f. prakt. Chemie*, xvi. (1877). *Zei*

A very excellent general account of the spectrophotometer and its applications in physiological chemistry is given by Lambling[1] in a paper, which the reader is advised to consult. The method consists essentially in measuring the diminution in intensity which a beam of light undergoes in its passage through a coloured solution, and in deducing the concentration of the solution from such a measurement. Given two rays of equal initial intensity, one of which is perceived directly by the observer, and the other after its passage through a coloured solution, what one has to do is to measure the relative intensity of the two rays. But such observations must be made not with white light, which is mixed light, but with homogeneous light; in other words, the rays from a particular part of the spectrum; hence the term spectrophotometry.

The amount of absorption varies for the same substance and the same region of the spectrum with the concentration and thickness of the layer of liquid examined. A double layer of the liquid would produce the same effect in absorbing light as a single layer of a liquid twice as concentrated.

The quantity of light absorbed, however, does not increase directly with the thickness or concentration of the coloured liquid. Suppose a luminous ray of intensity equal to 1 passing through a layer of coloured liquid of one unit's thickness, its intensity is reduced to $\frac{1}{n}$; when, however, this ray of diminished intensity passes through another similar layer, its intensity is diminished by $\frac{1}{n} \times \frac{1}{n} = \frac{1}{n^2}$, and after passing through m similar layers to $\frac{1}{n^m}$.

The co-efficient of extinction (Bunsen) ϵ of a coloured solution is the inverse of a number expressing the thickness of the layer of that solution which is necessary to reduce the intensity of the light to one tenth of its initial intensity. We have already seen if $I' =$ final intensity, $1 =$ initial intensity, and $m =$ thickness of layer, that

$$I' = \frac{1}{n^m}$$

From which we see that $$n^m = \frac{1}{I'} \qquad \text{(Equation 1).}$$

Converting these into logarithms:—

$$m \log n = -\mathrm{lo}'$$

Therefore $$\log n = -\frac{\log I'}{m} \qquad \text{(Equation 2).}$$

But by the definition of the co-efficient of extinction:—

$$I' = \frac{1}{10} \text{ and } m = \frac{1}{\epsilon}.$$

Therefore $$n^m = n^{\frac{1}{\epsilon}} \text{ and } \frac{1}{I'} = 10$$

If we put these values of n^m and $\frac{1}{I'}$ in equation 1 we get

$$n^{\frac{1}{\epsilon}} = 10$$

Which in terms of logarithms is:—

$$\frac{1}{\epsilon} \log n = \log 10 = 1$$

---

[1] *Arch. de Physiologie*, 1888, p. 1.

Therefore $\log n = \epsilon$.

Putting this value of $\log n$ into equation 2, we get:

$$\epsilon = -\frac{\log I'}{m}$$

And if $m =$ unity, $\epsilon = -\log I'$.

In other words, the co-efficient of extinction is obtained by taking the negative logarithm of the fraction which represents the final intensity of the light. Suppose, for instance, that a solution of oxyhæmoglobin observed in a layer 1 centimetre thick reduced the luminous intensity in the region of the β band to 0·225 of the original, then $\epsilon = -\log 0{\cdot}225$

$\qquad = \quad 0{\cdot}64782$

Suppose that C, C′, C″ . . . represent the respective concentrations of a series of solutions, and ϵ, ϵ', ϵ'' . . . the corresponding co-efficients of extinction, then: $\frac{C}{\epsilon} = \frac{C'}{\epsilon'} = \frac{C''}{\epsilon''} \quad . \;\; . \;\; . \quad = A$.

This constant A can be easily measured in a solution of known strength: it is called the absorptive power:

$$A = \frac{C}{\epsilon}$$

Therefore $C = A\epsilon$. In other words, the concentration of the solution (number of grams in 1 c.c. of solution) can be ascertained by multiplying the co-efficient of extinction by the constant A.

This method can also be applied to mixtures of two colouring matters in solution—*e.g.* hæmoglobin and oxyhæmoglobin—provided that the constant A is known for both substances in two regions of the spectrum. The co-efficient of extinction in the same two regions is then determined by observation. The formula is somewhat complicated, and the memoir already referred to must be consulted for this matter, as well as for other interesting suggestions relating to the examination of other animal pigments, as of the bile, urine, &c., by means of the spectrophotometric method.

The forms of spectrophotometer that have been invented for the determination of co-efficients of extinction are very numerous. I select for description one invented by Glazebrook, and described by Dr. Sheridan Lea in the 'Journal of Physiology.'[1] In principle it is the same as Hüfner's, but differs from it, in that the light from both sources is polarised, whereas in Hüfner's instrument the light from only one source is polarised. The light, then, from each of two sources is polarised by a nicol's prism, and then allowed to pass through a direct vision prism, whereby two adjacent superposed spectra are obtained, and these are observed through an eye-piece, in which is an analysing nicol. This eye-piece can be rotated on its axis, the amount of rotation being measured by a pointer which moves over a circle divided into degrees. The spectra are further observed through a narrow slit in the eye-piece, so that only a small piece of the spectrum is seen, the part, in fact, for which one is making the determination. Set the pointer of the eye-piece at 0°, and then rotate one of the polarising nicols until the spectrum formed by the light passing through it is eclipsed: now set the pointer at 90°, and rotate the other polarising nicol till the second spectrum is eclipsed. Then set the pointer in some intermediate position in which the two spectra are of equal brightness. Now let the solution of the pigment of unknown concentration C be introduced on the path of the light which forms one of the spectra. In order to produce equality of the spectra the pointer of the eye-piece must be rotated into a fresh position.

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[1] Vol. v. p. 239.

Now if θ be the angle through which the eye-piece was rotated from 0° in order to produce the original equality of the spectra, and θ' be the angle of rotation required to produce equality when the absorbing substance is interposed, our formula $C = A\epsilon$ becomes $C = 2A\log\frac{\tan\theta}{\tan\theta'}$.

Suppose, now, that the same be done with a solution of known concentration, C', and that θ'' be the angle of rotation required to produce equality when this solution is interposed in the path of the light from one source, then $C' = 2A\log\frac{\tan\theta}{\tan\theta''}$. Hence, $\frac{C}{C'} = \frac{\log\tan\theta - \log\tan\theta'}{\log\tan\theta - \log\tan\theta''}$ from which equation C can be calculated.

THE SPECTRO-POLARIMETER[1]

This instrument is one, in which a spectroscope and polarising apparatus are combined for the purpose of determining the concentration of solutions of substances which rotate the plane of polarised light. It was invented by E. v. Fleischl, for the estimation of diabetic sugar in urine. Its chief advantage is, that no difficulty arises of forming a judgment, as to the identity of two coloured surfaces, as in Soleil's saccharimeter, or of two shades of the same colour, as in Laurent's instrument. The light enters at the right-hand end of the instrument, is polarised by the nicol's prism *b*, and then passes through two quartz plates, *cc*, placed horizontally over one another. One of these plates is dextro-, the other lævorotatory, and they are of such a thickness (7·75 mm.) that the green rays between the E and *b* lines of the spectrum are circularly polarised through an angle of 90°, the one set passing off through the upper quartz to the left, the

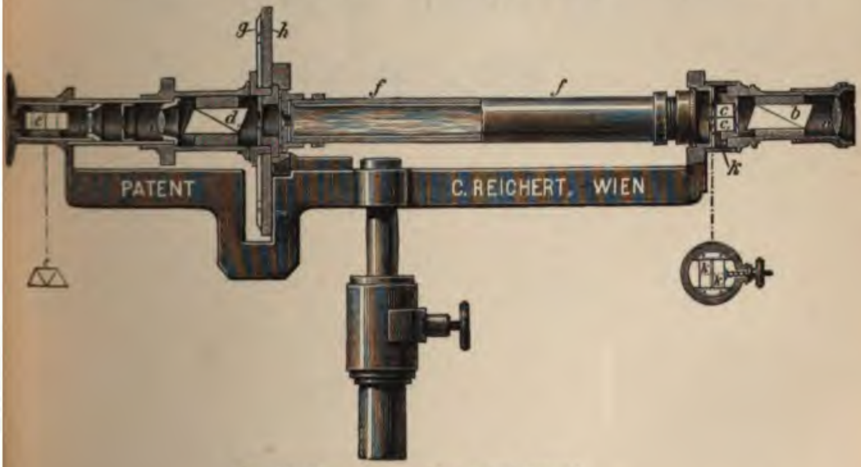

FIG. 30.—Spectropolarimeter of von Fleischl.

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[1] The following account of this instrument is taken from Dr. McKendrick's *Physiology*,

CHAPTER VI

INTRODUCTORY

THE chemical constituents of the animal body are exceedingly numerous; they consist of chemical elements, of inorganic compounds, and lastly of organic compounds. The organic compounds are the most numerous; some of them have a simple structure, but the greater number are very complicated.

The elements found in the body are carbon, hydrogen, nitrogen, oxygen, sulphur, phosphorus, fluorine, chlorine, silicon, sodium, potassium, calcium, magnesium, lithium, iron, and occasionally manganese, copper, and lead.

Of these very few occur in the free state; oxygen (to a small extent) and nitrogen are found dissolved in the blood. Hydrogen is found in the alimentary canal. Particles of carbon breathed in with the air may be found in the tissues of the lung. With some few exceptions such as these, the elements enumerated above are found combined with one another to form what are called compounds.

The compounds, or as they are sometimes termed in physiology, the **proximate principles**, found in the body are divided into (1) **mineral** or **inorganic** compounds, and (2) **organic** compounds or **compounds of carbon.**

The inorganic compounds present are water, peroxide of hydrogen, sulphuretted hydrogen, ammonia, various acids, and numerous salts (sodium chloride, calcium phosphate, &c.).

The organic compounds present are the various groups of alcohols and organic acids, and their compounds such as the fats; various derivatives of ammonia, for instance amides, amines, &c.; the aromatic bodies, and lastly the proteids, albuminoids, pigments, ferments, carbohydrates, and glucosides.

CHAPTER VII

INORGANIC COMPOUNDS

WATER (H_2O)

Water forms about 58·5 per cent. of the weight of the body; it occurs in different proportions at different ages, the proportion becoming smaller as life advances. In an infant the amount present is 66·4 per cent. (Bischoff).

The following table from Beaunis [1] gives the proportion of water in various solids and fluids of the body, in parts per 1000.

Enamel	2	Grey matter of brain	858
Dentine	100	Vitreous humour	987
Bone	486	Blood	791
Fat	299	Bile	864
Elastic tissue	496	Milk	891
Cartilage	550	Liquor sanguinis	901
Liver	693	Chyle	928
Spinal cord	697	Lymph	958
White matter of brain	700	Serum	959
Skin	720	Gastric juice	973
Brain	750	Intestinal juice	975
Muscle	757	Tears	982
Spleen	758	Aqueous humour	986
Thymus	770	Cerebro-spinal fluid	988
Connective tissue	796	Saliva	995
Kidneys	827	Sweat	995

An adult takes into the body in the form of food (solid and liquid) about 2,500 c.c. of water per diem. A small quantity is formed in the body from the combination of hydrogen and oxygen, and thus a larger quantity is excreted than is actually taken in. On the average, the daily excretions contain about 2,600 c.c. of water.

A diminution of the quantity of water leads to the sensation known as thirst; in frogs, when they have lost 30 per cent. of their weight of water, death ensues.

A great increase in the quantity of water is harmful, as it increases tissue waste, and carries off a large amount of the solids, especially the saline solids of the body, in solution.

[1] J

Injection of water into the circulation in large quantities causes death, as it dissolves the hæmoglobin from the corpuscles and so interferes with respiratory functions (Picot).[1]

In starving animals (pigeons) the relation of water to solids only shows important changes when the total body weight is diminished by 34 per cent. and the animal has taken no solid or liquid food for 133 hours. The relation in some organs (heart, kidneys, thoracic muscles, alimentary tract, blood, brain, and lungs) even then undergoes little or no change; in others (thigh muscles, and bones) the water is increased; and in a third category (spleen, pancreas, liver) the water is diminished (Lukjanow).[2]

PEROXIDE OF HYDROGEN (H_2O_2)

C. Wurster[3] uses paper soaked with a solution of tetramethylparaphenylenediamine as a delicate test for active oxygen, a blue-violet colour being formed. The development of this colour by means of certain tissues, and fluids of the body (skin, sweat, &c.), is believed to be due to the evolution of active oxygen from peroxide of hydrogen, present in those parts. Peroxide of hydrogen coagulates albumin, and Wurster considers it possible that its presence may explain such phenomena as the coagulation of the blood and of muscle; and by its action on the hæmoglobin of the blood various other pigments, such as those of the skin and hair, may be produced. In want of further proofs of these and other functions assigned to peroxide of hydrogen, we must accept all such conclusions with the greatest possible reserve.

SULPHURETTED HYDROGEN (H_2S)

This gas occurs free in the alimentary canal, being formed by putrefactive processes which occur there.

AMMONIA

This also is formed in small quantities during these putrefactive processes. It, however, soon enters into combinations to form salts or organic compounds. Ammonia also occurs in urine, especially if it has been allowed to undergo putrefaction within or without the body.

The mineral salts and organic compounds in which ammonia occurs will be described under other headings.

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rend. 1874, p. 62.

[2] *Zeit. physiol. Chem.* xiii. 339.

ichte deutsch. Chem. Gesellsch. xix. 3195; xx. 268, 1033.

ACIDS

Free hydrochloric acid occurs in the gastric juice. Free sulphuric acid in the so-called saliva of certain gasteropods (*Dolium galea*, &c.).[1]

The acids found in the body are, as a rule, not free, but combined with bases to form salts (chlorides, sulphates, &c.).

Free carbonic acid is found in small quantities dissolved in the fluid and solid tissues of the body.

SALTS

The chief salts found, are the chlorides of sodium and potassium, the sulphates of the same metals, phosphates of sodium, potassium, calcium, and magnesium, and the carbonates of sodium and calcium.

Bone and similar tissues like dentine and enamel are chiefly rich in calcium salts, especially the phosphate.

Other solid tissues, except the lungs, are especially rich in potassium salts. In fluids (milk excepted) the most abundant salt is sodium chloride.

Enumerations of the various saline constituents will be given when we consider the fluids, tissues, and organs themselves. The following general tables, however, compiled by Beaunis[2] may appropriately be quoted here. The figures give percentage quantities of mineral matters in the ash.

Tissue	Bone	Calf-muscles	Brain	Liver	Lungs	Spleen
Analyst	Heintz	Staffel	Breed	Oidtmann	C. Schmidt	Oidtmann
Sodium chloride .	—	10·59	4·74	—	13·0	—
Soda . . .	—	2·35	10·69	14·51	19·5	44·33
Potash . . .	—	34·40	34·42	25·23	1·3	9·60
Lime . . .	37·58	1·99	0·72	3·61	1·9	7·48
Magnesia . . .	1·22	1·45	1·23	0·20	1·9	0·49
Ferric oxide . .	—	—	—	2·74	3·2	7·28
Chlorine . . .	—	—	—	2·58	—	0·54
Fluorine . . .	1·66	—	—	—	—	—
Phosphoric acid .	53·31	48·13	48·17	50·18	48·5	27·10
Sulphuric „ .	—	—	0·75	0·92	1·4	2·54
Carbonic „ .	5·47	—	—	—	—	—
Silicic „ .	—	0·81	0·12	0·27	—	0·17

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[1] Boedeker, *Pogg. Ann.* vol. xciii. p. 614. *Journ. prakt. Chem.* vol. lxiii. p. 170. Panceri and de Luca, *Compt. rend.* vol. lxv. pp. 577, 712. These three observers found from 2·5 to 3·5 per cent. of free sulphuric acid in this remarkable secretion.

[2] I am indebted for the reference to these tables to Dr. McKendrick's *Textbook on Physiology*. The subsequent remarks on the individual salts are very largely a *résumé* from the same work.

Fluid . . .	Blood	Serum	Blood-clot	Lymph	Urine	Milk	Bile	Excrements
Analyst . . .	Verdeil	Weber	Weber	Dahnhardt	Porter	Wilderstein	Rose	Porter
Sodium chloride	58·81	72·88	17·36	74·48	67·26	10·73	27·70	4·33
Potassium „	—	—	29·87	—	—	26·33	—	—
Soda . .	4·15	12·93	3·55	10·35	1·33	—	36·73	5·07
Potash . .	11·97	2·95	22·36	3·25	13·64	21·44	4·80	6·10
Lime . .	1·76	2·28	2·58	0·97	1·15	18·78	1·43	26·40
Magnesia . .	1·12	0·27	0·53	0·26	1·34	0·87	0·53	10·54
Ferric oxide .	8·37	0·26	10·43	0·05	—	0·10	0·23	2·50
Phosphoric acid	10·23	1·73	10·64	1·09	11·21	19·00	10·45	36·03
Sulphuric „	1·67	2·10	0·09	—	—	2·64	6·39	—
Carbonic „	1·19	4·40	2·17	8·20	—		11·26	—
Silicic „	—	0·20	0·42	0·42	4·06		0·36	3·13

Sodium Chloride and Potassium Chloride.—Probably 200 grammes may be taken as an average amount of sodium chloride (common salt) in the adult human body. It is a most important food, and from 15–20 grammes are daily excreted in the urine, and smaller amounts in the sweat and fæces. If potassium chloride be substituted in the food for the sodium salt, disturbances arise from deficiency of the latter. The tissues, however, retain common salt very tenaciously, so that during a dietary devoid of salt, it disappears slowly from the urine.

During its passage through the body, it facilitates the absorption of proteid food, and increases tissue metabolism. Probably a small amount is decomposed yielding its chlorine to potassium, the chloride of which metal is indispensable to tissues like muscle. The following table gives the relative amounts of the two salts in parts per 1000.

	NaCl	KCl		NaCl	KCl
Blood	2·70	2·05	Pancreatic juice (from temporary fistula) .	7·35	0·02
Blood corpuscles . .	—	3·67	Gastric juice . . .	1·45	0·55
Plasma	5·54	—	Bile	5·33	0·28
Lymph	5·67	—	Milk	0·87	2·13
Chyle	5·84	—	Urine	11·00	4·50
Pancreatic juice (from permanent fistula) .	2·50	0·93			

Other Sodium and Potassium Salts.—Bunge found that the soda salts are more abundant in embryonic and early life than in adult life. This is illustrated by the following table :—

	Na_2O	K_2O		Na_2O	K_2O
Rabbit's embryo .	2·183	2·605	Cat, 29 days old .	2·292	2·684
Rabbit, 14 days old .	1·630	2·967	Dog, 4 „ „ .	2·589	2·667
Kitten, 1 day „ .	2·666	2·691	Adult mouse . .	1·700	3·280
Cat, 19 days „ .	2·285	2·790			

This fact is probably due to the larger amount of cartilage (rich in soda salts), and the smaller amount of muscle (rich in potash salts) in early life, as compared with the adult condition.[1]

Sodium phosphate (Na_3PO_4), acid sodium phosphate (Na_2HPO_4), and acid potassium phosphate (K_2HPO_4) are found in the urine, the latter salts causing the acidity of that secretion. Phosphates of sodium and potassium also occur in the blood and tissues. Sodium carbonate (Na_2CO_3) and bicarbonate ($NaHCO_3$) occur in the food, but are chiefly formed from the salts of vegetable acids (tartaric, citric, &c.). They occur in the blood, and carry the carbonic acid from the tissues to the lungs.

Sodium sulphate (Na_2SO_4) and potassium sulphate (K_2SO_4) exist in small quantities in the body; they are partly introduced with the food, but chiefly formed by the oxidation of proteids and other organic substances containing sulphur.

Ammonium Salts.—Minute traces of ammonium chloride are found in the urine; ammonium carbonate is formed from urea in decomposing urine. The urine of reptiles, and birds is largely composed of ammonium urate. Small quantities of this salt, and also of ammonio-magnesic phosphate, are found in human urine.

Calcium Salts.—About three-quarters of the total mineral solids in the body consist of calcium phosphate, $Ca_3(PO_4)_2$; this is because of the great preponderance of this salt in bone. Other calcium salts occurring in bone, dentine, and enamel are the carbonate, sulphate and fluoride. Calcium phosphate, urate and oxalate are found in the urine. Most tissues contain small quantities of the phosphate and carbonate. Egg shells, the shells of crustacea, coral, and otoliths consist chiefly of carbonate of lime.

Magnesium Salts.—Magnesium phosphate ($Mg_3(PO_4)_2$) occurs in the tissues, along with the calcium phosphates ($Ca_3(PO_4)_2$ and $CaH_4(PO_4)_2$), but in smaller amount. It occurs also in the urine; ammonio-magnesium or triple phosphate ($NH_4MgPO_4+6H_2O$) is also often found in decomposing urine. Magnesium palmitate and stearate are found in the fæces.

Iron is an important constituent of the blood-pigment. The blood of an adult contains 3 grammes of iron. Small quantities are found in other liquids of the body (chyle, lymph, bile, milk, urine, gastric juice); it is also contained in the black pigment of the skin and hair, and of melanotic sarcomata. A small quantity of ferric sulphide is found in the fæces, and small quantities of iron are found in both liver and spleen.

[1] Bunge, *Zeit. physiol. Chem.* xiii. 399.

Other Metals. — Copper is found in two proximate principles, hæmocyanin, the blue pigment of the blood of many invertebrates (crustacea, cuttle fishes, scorpions, &c.), and in the pigment turacin of birds' feathers. Small quantities of this metal, and also of aluminium, manganese and lead, may occur accidentally in other parts, being taken in with the food, and not excreted at once with the fæces, but deposited in some tissue or organ. Drugs and poisons (mercury, arsenic, &c.) may be similarly deposited.

Silicon.—A minute quantity of silica exists in the blood, urine, bones, hair, and other parts.

Phosphates.—The amount of phosphoric acid given in analyses of the ash of animal structures is not always correct, since a certain quantity is obtained during the process of incineration from the decomposition of organic compounds, which like lecithin contain phosphorus.

The phosphoric acid which occurs in mineral compounds in the body is derived partly from the food, and partly from the metabolism of lecithin and nuclein. It unites with soda, potash, lime and magnesia to form the various phosphates already alluded to. An adult man eliminates by the kidneys 2·5–3·5 grammes of phosphoric acid daily. Carnivora eliminate phosphates chiefly by the kidneys, herbivora chiefly with the fæces.

Carbonates.—The presence of carbonates in the ash of animal matters is partly derived from the decomposition of organic compounds. Alkaline carbonates and bicarbonates are however found in blood, urine, lymph, saliva, &c.

Sulphates.—These also may be partly formed during the process of incineration from proteids, and other organic compounds containing sulphur. The sulphuric acid in the urine is partly combined as ordinary sulphates, partly as ethereal sulphates. It is derived to a small extent from the food, but chiefly from the metabolism of proteids, the amount of sulphuric acid and urea in the urine running parallel with one another.

CHAPTER VIII

THE SIMPLER ORGANIC PROXIMATE PRINCIPLES

IT was at one time supposed that the organic compounds differed f the inorganic, in the fact that it was not possible for the chemis make them artificially from their elements. Many of the orga constituents of the body have however been produced in the laborat since that time.[1]

Organic compounds are now regarded as the compounds of carb this definition would however include carbonates, which we have alre considered with inorganic substances. Schorlemmer describes orga chemistry, as the chemistry of the hydrocarbons and their derivati

Carbon is a tetrad element; its atomic weight is 12 (11·97).

THE PARAFFINS AND THEIR DERIVATIVES

The simplest hydrocarbon known is marsh gas or *methane*; formula is CH_4. This is the first member of the series known as paraffins. The paraffins differ from one another by CH_2; the sec member of the series, *ethane*, has the formula C_2H_6; the third, C_3 and so on. The typical formula for the series is therefore C_nH The lowest members of the series are gaseous, the next fluid, and higher members form the solid or hard paraffins.

By replacing an H of the hydrocarbon by hydroxyl (OH), a co pound of the nature of a hydrate is formed. In the case of metha the formula for this hydrate will be CH_3.OH; in the case of eth C_2H_5.OH; and so on throughout the series. These hydrates di from the metallic hydrates, like potassium hydrate (K.OH) or sodi hydrate (NaOH), by the fact that the hydroxyl is combined, not w a metal, but with a group of atoms called a radicle; in the case methane with CH_3 which is called *methyl*; in the case of ethane w C_2H_5 which is called *ethyl*, and so throughout the series. These orga hydrates are called *alcohols*, the first *methylic*, the second *ethylic* common alcohol, and so on.

There are, as we shall see, other series of alcohols in addition those derived from this paraffin series. This first and simplest fa of alcohols are known as the *monatomic alcohols*; that is, they contain only one hydroxyl group, and the radicle (methyl, ethyl, &c.) is therefore monovalent, like the metals hydrogen, sodium, potassium, &c.

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[1] The synthesis of urea by Wohler in 1828 was the first step in this direction.

Another group of substances called *ethers* are derived from the alcohols by treating them with dehydrating agents; thus :—

$$\underset{\text{ethyl alcohol}}{2(C_2H_5)HO}-H_2O=\underset{\text{ethyl ether}}{(C_2H_5)_2O}$$

The ethers of the monatomic alcohol are seen to be analogous to the oxides of the monad metals (H_2O, Na_2O, &c.).

By oxidation of the alcohols, two atoms of hydrogen are removed, and another group of substances called *aldehydes* are obtained; thus :

$$\underset{\text{ethyl alcohol}}{(C_2H_5)OH}+O=\underset{\text{ethyl aldehyde}}{CH_3.COH}+H_2O$$

On further oxidation still, the two atoms of hydrogen removed, to form the aldehyde, are replaced by one atom of oxygen[1]; thus :—

$$\underset{\text{ethyl aldehyde}}{CH_3.COH}+O=\underset{\text{acetic acid}}{CH_3.COOH}$$

In this way, an *acid* is ultimately formed from the alcohol, and the series of acids derived from the series of monatomic alcohols are monobasic[2]; they constitute what is known as the fatty acid series. These acids combined with metallic bases like soda or potash form compounds known as *soaps*; when combined with organic bases, such as glycerine, they form what are known as *fats*.

The following table represents in a compact way the different classes of compounds derived from this group of paraffins.

Hydrocarbon	Radicle	Alcohol	Aldehyde	Acid
C_nH_{2n+2}	C_nH_{2n+1}	$C_nH_{2n+1}HO$	$C_nH_{2n}O$	$C_nH_{2n}O_2$
CH_4 (methane)	CH_3 (methyl)	$CH_3.HO$	—	CH_2O_2—formic acid
C_2H_6 (ethane)	C_2H_5 (ethyl)	$C_2H_5.HO$	C_2H_4O	$C_2H_4O_2$—acetic „
C_3H_8 (propane)	C_3H_7 (propyl)	$C_3H_7.HO$	C_3H_6O	$C_3H_6O_2$—propionic „
C_4H_{10} (butane)	C_4H_9 (butyl)	$C_4H_9.HO$	C_4H_8O	$C_4H_8O_2$—butyric „
C_5H_{12} (pentane)	C_5H_{11} (amyl)	$C_5H_{11}.HO$	$C_5H_{10}O$	$C_5H_{10}O_2$—valerianic „
C_6H_{14} (hexane)	C_6H_{13}	$C_6H_{13}.HO$	$C_6H_{12}O$	$C_6H_{12}O_2$—caproic „
⋮	⋮	⋮	⋮	⋮
$C_{16}H_{34}$ (hecdecane)	—	—	—	$C_{16}H_{32}O_2$—palmitic „
$C_{18}H_{38}$	—	—	—	$C_{18}H_{36}O_2$—stearic „

It is well known that there are alcohols having the same percentage composition, but which differ in their products of oxidation. For example, there are two propyl alcohols both with the formula C_3H_8O ; one, yielding on oxidation, as above shown, an aldehyde and then propionic acid ; the second, yielding a substance called *acetone*, the first of a homologous series of compounds called *ketones*. This is explained as follows :

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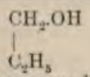
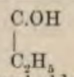
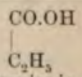
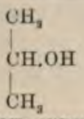
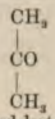
[1] This production of acetic acid from alcohol is usually effected by an organised ferment called *bacterium aceti*.

[2] Monobasic means that one atom of the hydrogen is replaceable by a monad metal (K or Na), or by a monad radicle, in the latter case forming what is known as a compound

Primary propyl alcohol (the one which yields an aldehyde) contains a monatomic group of atoms $CH_2.OH$; the other, or secondary propyl alcohol, contains a diatomic group $CH.OH$. In oxidation of the primary alcohol, H_2 is removed from the group $CH_2.OH$; in oxidation of the secondary alcohol, HH is removed from the group $CH.OH$. Thus :—

Alcohol	Intermediate product	Acid
 $\begin{array}{l}CH_2.OH\\ \vert\\ C_2H_5\end{array}$ Primary propyl alcohol	 $\begin{array}{l}C.OH\\ \vert\\ C_2H_5\end{array}$ propyl aldehyde	 $\begin{array}{l}CO.OH\\ \vert\\ C_2H_5\end{array}$ propionic acid
 $\begin{array}{l}CH_3\\ \vert\\ CH.OH\\ \vert\\ CH_3\end{array}$ Secondary propyl alcohol	 $\begin{array}{l}CH_3\\ \vert\\ CO\\ \vert\\ CH_3\end{array}$ propyl ketone or acetone	The ketone when further oxidised cannot give any C_3 acid because the group $C.OH$ is wanting. It yields CO_2, H_2O, and acetic acid

In like manner we have four butyl alcohols, and the number of higher alcohols, which are theoretically possible, increases rapidly as we ascend the scale.

Compounds like this having the same empirical formula and molecular weight, but differing in certain reactions and physical properties owing to the difference in their chemical constitution, are called *isomeric*. Substances having the same percentage composition, but different molecular weights, are called *polymeric*. Thus aldehyde (C_2H_4O) and paraldehyde ($C_6H_{12}O_3$) are polymeric ; so are lactic acid ($C_3H_6O_3$) and dextrose ($C_6H_{12}O_6$).

The group of hydrocarbons which we have just considered forms a homologous series with the general formula C_nH_{2n+2}, and the lowest term of these series is the simplest hydrocarbon known, namely, methane or marsh gas. There are in addition to these paraffins, however, other series of non-saturated hydrocarbons, each of which may be the basis of a group of chemical substances. The general formulæ for these series of paraffins are C_nH_{2n}, C_nH_{2n-2}, C_nH_{2n-4}, C_nH_{2n-6}, C_nH_{2n-8}, and so forth.

The series of paraffins starting with methane (CH_4) forms the basis of the ordinary monatomic alcohols, and the corresponding fatty acids.

The series of paraffins with the general formula C_nH_{2n} starts with ethene, ethylene or olefiant gas C_2H_4, and forms the basis of a series of alcohols, which are called glycols. These, like the first series of alcohols, are hydrates, i.e. compounds of hydroxyl with organic radicles, only in the case of the glycols there are two molecules of hydroxyl united to the radicle instead of one as in the monatomic alcohols. In other words, the glycols are diatomic alcohols.

Looked at in another way, a monatomic alcohol may be regarded as built on the type of one molecule of water, in which one atom of hydrogen is replaced by the radicle. Thus :—

$$\underset{\text{water}}{H.OH} \qquad \underset{\text{methyl alcohol}}{CH_3.OH}$$

A diatomic alcohol is built on the type of two molecules of water in which HH is replaced by the radicle.

$$\underset{\text{water}}{\begin{matrix}H.OH\\H.OH\end{matrix}} \qquad \underset{\text{ethyl glycol}}{C_2H_4\begin{cases}OH\\OH\end{cases}}$$

We compared the monatomic alcohols to the hydrates of the monad metals, like sodium hydrate (Na.OH) or potassium hydrate (K.OH).

We may compare the diatomic alcohols or glycols to the hydrates of the dyad metals, like calcium hydrate $Ca\begin{cases}OH\\OH\end{cases}$ or magnesium hydrate $Mg\begin{cases}OH\\OH\end{cases}$.

From the glycols[1] or diatomic alcohols, acids are obtained by oxidation; H_2 is removed, and replaced by O ; an acid is formed which is called glycolic acid,[1] and is the first of a series of acids similarly derived from the series of alcohols.

But the glycols, as we have seen, are diatomic, and so the operation can be repeated, and another H_2 removed and replaced by O. This gives us a second series of acids of which oxalic acid is the first member.

The following table represents in a compact way the different classes of compounds derived from the hydrocarbons with general formula C_nH_{2n}.

Hydrocarbon	Alcohol (diatomic)	Acid. 1st series (monobasic)	Acid. 2nd series (dibasic)
C_nH_{2n}	$C_nH_{2n}(HO)_2$	$C_nH_{2n}O_3$	$C_nH_{2n-2}O_4$
		[CH_2O_3 carbonic acid]	
C_2H_4 ethene	$C_2H_4(HO)_2$ ethene-glycol	$C_2H_4O_3$ glycolic ,,	$C_2H_2O_4$ oxalic acid
C_3H_6 propene	$C_3H_6(HO)_2$ propene ,,	$C_3H_6O_3$ lactic ,,	$C_3H_4O_4$ malonic ,,
C_4H_8 butene	$C_4H_8(HO)_2$ butene ,,	$C_4H_8O_3$ oxybutyric ,,	$C_4H_6O_4$ succinic ,,
C_5H_{10} amylene	$C_5H_{10}(HO)_2$ amylene ,,	$C_5H_{10}O_3$ valero-lactic ,,	$C_5H_8O_4$ pyrotartaric or glutaric ,,
C_6H_{12} hexene	$C_6H_{12}(HO)_2$ hexene ,,	$C_6H_{12}O_3$ leucic ,,	$C_6H_{10}O_4$ adipic ,,
	⋮	⋮	⋮

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[1] Glycol must be carefully distinguished from an entirely different substance with a similar name, glycocoll, or glycocine. Glycolic acid is also similar in name but very different in nature from glycocholic acid, one of the acids of the bile.

The next group of hydrocarbons on our list consists of those with the general formula C_nH_{2n-2}. This is illustrated by the compound called acetylene, which has the formula C_2H_2. The fourth series (C_nH_{2n-4}) is illustrated by terebinthene $C_{10}H_{16}$; the fifth (C_nH_{2n-6}) is illustrated by benzene C_6H_6; the sixth series (C_nH_{2n-8}) by cinnamene C_8H_8, &c. The differences between the benzene derivatives and the rest are so marked that they are classed as *aromatic compounds.*

In a similar way there are other groups of alcohols; the next series is that of the triatomic alcohols, that is, those like glycerine or glycero built on the type of three molecules of water: i.e. a radicle is united to three molecules of hydroxyl. $C_3H_5(HO)_3$ is the formula for glycerine Tetratomic alcohols are instanced by erythrite $C_4H_6(HO)_4$; and hexatomic alcohols by mannite $C_6H_8(HO)_6$.

These different families of alcohols may be contrasted with one another in the following tabular way :—

ALCOHOLS

Monatomic On type of 1 mol. water	Diatomic On type of 2 mol. water	Triatomic On type of 3 mol. water	Tetratomic On type of 4 mol. water	Hexatomic On type of 6 mol. water
H.OH	H.OH H.OH	H.OH H.OH H.OH	H.OH H.OH H.OH H.OH	H.OH H.OH H.OH H.OH H.OH H.OH
Example $CH_3.OH$ Methylalcohol	Example $C_2H_4\left\{\begin{matrix}OH\\OH\end{matrix}\right.$ Ethene alcohol or Ethyl glycol	Example $C_3H_5\left\{\begin{matrix}OH\\OH\\OH\end{matrix}\right.$ Glycerine or Glycerol	Example $C_4H_6\left\{\begin{matrix}OH\\OH\\OH\\OH\end{matrix}\right.$ Erythrite	Example $C_6H_8\left\{\begin{matrix}OH\\OH\\OH\\OH\\OH\\OH\end{matrix}\right.$ Mannite

From all these alcohols, many different series of acids are derived It would, however, lead us too far to give tables of all the derivatives of the higher alcohols. The sketch given of the general plan of the homologous series derived from monatomic and diatomic alcohols must suffice. We have, however, merely to repeat the process in a somewhat more complicated way for the higher alcohols. Thus from the triatomic alcohols a series of acids commencing with glyceric and tartronic acids are derived.

The carbohydrates (starches, sugars) are derivatives of the hexatomic alcohol, mannite.

We have seen how in some of the simpler organic compounds polymerism and isomerism may occur. They occur, as one would naturally expect, to a far greater extent in the substances with more complex formulæ.

There is, lastly, a series of substances in which the carbon of the hydrocarbon is non-saturated; thus the formula for allyl alcohol is

$$\left.\begin{matrix}C_3H_5\\H\end{matrix}\right\}O$$

in which it is evident two atomicities are unsatisfied. Related to such alcohols are the acids of the acrylic series, in the same manner as the fatty acids are related to the monatomic alcohols. Thus :—

Alcohol	*Acid*
$\left.\begin{matrix}C_2H_5\\H\end{matrix}\right\}O$	$\left.\begin{matrix}C_2H_3O\\H\end{matrix}\right\}O$
[ethyl alcohol]	[acetic acid]
$\left.\begin{matrix}C_3H_5\\H\end{matrix}\right\}O$	$\left.\begin{matrix}C_3H_3O\\H\end{matrix}\right\}O$
[allyl alcohol]	[acrylic acid]

The acids having the general formula $C_nH_{2n-2}O_2$ are

$C_3H_4O_2$	Acrylic	acid
$C_4H_6O_2$	Crotonic	„
$C_5H_8O_2$	Angelic	„
⋮		
$C_{18}H_{24}O_2$	Oleic	„
⋮		

Having thus described the general chemical characteristics of these groups of hydrocarbons with their derivatives, we pass on to enumerate the members of those groups that occur in the body.

Hydrocarbons.—*Methane* (CH_4) is the only member of this series found in the body. It occurs mixed with other gases in the intestinal canal.

Alcohols.—*Ethylic alcohol* is a constituent of the fermented liquors used as beverages. It may, however, be formed in small quantities by a process of fermentation occurring in the intestinal canal. Thus it may be found in small quantities in the urine even when it is absent from the food.[1]

Cholesterin ($C_{26}H_{43}.HO$) is a monatomic alcohol, and though not one of the series beginning with methyl and ethyl alcohols, it is convenient to include it in this place. It crystallises either without water (from anhydrous ether) or with one molecule of water of crystallisation (from a mixture of alcohol and water). It occurs in blood corpuscles, nervous tissues, and bile.

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[1] W. H. Ford, *Trans. Internat. Med. Congress*, Washington, 1887, vol. iii. p. 293.

Phenol or carbolic acid ($C_6H_5.OH$) may also be regarded as an alcohol; it is found in small quantities usually in combination as an ethereal sulphate in fæces and urine; it may, however, be more conveniently classed with the aromatic substances.

Glycerine is found in combination with fatty acids to form fats.

It is liberated from the fats during digestion, and may thus be found free in the alimentary canal. It may there be further decomposed into various acids, especially propionic acid, or after absorption may undergo complete combustion, forming carbonic acid and water. Beneke considers that the phosphoric acid liberated from the phosphates of the food unites with it, to form glycero-phosphoric acid which is a stage in the synthesis of lecithin. The relation of glycerine to the glycogen of the liver will be fully discussed in connection with that organ.

Cetyl alcohol $(C_{16}H_{33})HO$ is found combined with palmitic acid in spermaceti.

Cerotyl alcohol $(C_{27}H_{55})HO$ is contained in Chinese wax.

Melicyl alcohol $(C_{30}H_{61})HO$ is found in beeswax.

Various other alcohols, or compounds of them, are found in various vegetable oils and other products.

Aldehydes and Ketones.—*Acetone* may be found in the blood and urine in minute quantities even in health. This is increased in certain diseases, especially diabetes. There is, however, some uncertainty as to whether it occurs in the free condition, and the question will be fully discussed under Diabetes.

The carbohydrates are derivatives of mannite. The glucoses are aldehydes of that alcohol, and the other groups of carbohydrates (saccharoses and amyloses) are derived from the glucoses.

Fatty acids.—*Formic acid.*—This has been described as present in small quantities in spleen, pancreas, thymus, muscle, and brain. In leucocythæmia, it is said to be also found in the blood, urine, sweat, and marrow.

As its name implies, it is obtained from the bodies of ants. It is the substance also which gives, in all probability, the acid reaction to the blood of certain insects. It is a colourless liquid of strong odour. It volatilises at 100° C. without residue.

Acetic acid may be present in small quantities in bile and sweat, in the stomach and intestine as the result of fermentative changes. It is the chief acid contained in vinegar. It has a characteristic odour, a very sour taste ; it is volatile without residue. It forms transparent crystals which melt at 17° C.

Propionic acid occurs occasionally in sweat, in fermenting diabetic urine, in the blood in leucocythæmia, and in the vomit in cases of cholera. It volatilises at 142° C. ; its odour is like that of acetic acid.

Butyric acid is found in the sweat, fæces, urine, decomposing organic matter, in the sputum in gangrene of the lung, &c. It occurs with lactic acid, and is a further stage in what is known as the lactic acid fermentation. It volatilises at 160° C.

Butyric acid combined with glycerine to form a fat or glyceride is contained in milk.

Valerianic acid.—Either this acid or its ammonium salt is found in decomposing organic matter ; it may also be found in the urine and fæces in certain diseases (small-pox, typhus, acute yellow atrophy of the liver). It volatilises at 175° C.

Caproic acid may be occasionally found in sweat, and in fæces ; it occurs as a glyceride in butter. It volatilises at 202° C.

Caprylic acid ($C_8H_{10}O_2$; crystallises at 12° C.) and *capric acid* $C_{10}H_{20}O_2$; fusible at 70° C.) may occur in minute quantities in combination with glycerine in butter.

Palmitic and stearic acids are much higher in the series, and these, like the paraffins from which they are derived, are solid at the ordinary atmospheric temperature. They are found combined with glycerine, to form the chief solid fats of adipose tissue, and also occur in the fat of milk (cream). Palmitic acid is occasionally met with in pus, tubercles, and the sputum in gangrene of the lung. Combined with mineral bases to form soaps, they are found in small quantities during the digestion of fats in the alimentary canal, and also in the blood and lymph.

Acids related to the Glycols.—(*a*) *The Glycolic acid series.*—The first member of the group, *carbonic acid*, differs from the others in being dibasic. The next, *glycolic acid*, does not occur in the body, but is of interest as glycocine can be derived from it, by the substitution of NH_2 for one of the hydroxyls it contains. *Lactic acid*, of which there are three isomerides, occurs in many tissues of the body, and as a result of fermentative changes in milk. It will be more fully described under muscle. It may occur in the urine after extirpation of the liver. *Oxybutyric acid* generally occurs in diabetic urine. *Leucic acid* is related to the substance leucin, a result of the decomposition of proteids.

(*b*) *The Oxalic acid series.*—*Oxalic acid* occurs in the urine as oxalate of lime, where it is deposited as octahedral or dumb-bell crystals. Its relations to urea and uric acid will be discussed under urine. *Succinic acid* has been detected in the urine, after food containing malic acid or asparagine has been taken. Small quantities have been discovered in spleen, thymus, thyroid, hydrocele fluid, &c. Traces of this acid (and also of glycerine) are formed during the alcoholic fermentation of dextrose by means of yeast. Succinic acid ($C_4H_6O_4$) is closely related in composition to three acids contained in many vegetable foods, viz. malic acid ($C_4.H_6O_5$), tartaric acid ($C_4H_6O_6$), and citric acid ($C_6H_8O_7$).

Acids of the Acrylic Series.—*Acrylic acid* itself ($C_3H_4O_2$) is obtained by the oxidation of *acrolein* (C_3H_4O), the aldehyde of allyl alcohol. Acrolein is also produced by the removal of two molecules of water from glycerine ($C_3H_8O_3 - 2H_2O - C_3H_4O$).

Crotonic acid occurs in croton oil.

Angelic acid occurs in croton oil, and angelica root. Its aldehyde occurs in essential oil of chamomile.

Erucic acid ($C_{22}H_{42}O_2$), a high term of the series, is found in rapeseed oil.

Oleic acid ($C_{18}H_{34}O_2$) is more important to the physiologist, as it occurs not only in vegetable oils (almond oil, olive oil, &c.) but also in the glyceride *olein*, an important constituent of the fat of adipose tissue, and of milk.

Amido-acids.—These are acids derived from the fatty acids, by replacing one or more hydrogen atoms by the radicle amidogen (NH_2). This important group of substances, which includes leucine, tyrosine, glycocine, taurine, creatine, &c., will be more conveniently dealt with in connection with the nitrogenous proximate principles of the body.

The Fats.—The fat of adipose tissue is a mixture of the glyceric ethers, or glycerides of palmitic, stearic, and oleic acids variously mixed together.

In cream there are in addition small quantities of glycerides of fatty acids lower in the series.

Their chemical characteristics will be more fully described in connection with adipose tissue and milk.

Small quantities of fat are, however, found in other parts. The table on the next page from Gorup-Besanez gives the percentage of fat in the organs and fluids of the human body.

Moleschott found that in a man 30 years of age, weighing 64 kilogrammes, about 2·5 per cent. of the body weight was composed of fat. Burdach gives an average of 5 per cent. It need hardly be said that it varies immensely.

Sweat	0·001	Cartilage	1·3
Vitreous humour	0·002	Bone	1·4
Saliva	0·02	Crystalline lens	2·0
Lymph	0·05	Liver	2·4
Synovia	0·06	Muscles	3·3
Liquor amnii	0·06	Hair	4·2
Chyle	0·2	Brain	8·0
Mucus	0·3	Egg	11·6
Blood	0·4	Nerves	22·1
Bile	1·4	Adipose tissue	82·7
Milk	4·3	Marrow	96·0

Tripalmitin $C_3H_5(O.C_{16}H_{31}O)_3$ and *tristearin* $C_3H_5(O.C_{18}H_{35}O)_3$ are the solid fats of the body, they are held in solution at the body temperature by *triolein* $C_3H_5(O.C_{18}H_{33}O)_3$. *Trimargarin* is a mixture of the two first-named fats. *Tributyrin* $C_3H_5(O.C_4H_7O)_3$ is found in butter. *Trivalerin* $C_3H_5(O.C_5H_9O)_3$ exists in the oil of marine animals like the seal. *Tricaproin* $C_3H_5(O.C_6H_{11}O)_3$, *tricaprylin* $C_3H_5(O.C_8H_{15}O)_3$, and *tricaprin* $C_3H_5(O.C_{10}H_{19}O)_3$ are found in milk and butter.

In the decomposition of fat, we may find propionic, acetic, and formic acid, which are absent from the fat in the fresh condition. This occurs when a fat becomes rancid, and is doubtless produced also by the action of putrefactive organisms in the alimentary canal. This is really a process of oxidation : the way in which a lower term of the series is thus produced may be illustrated by the following equations :—

$$\underset{\text{[propionic acid]}}{C_3H_6O_2} + O_3 = \underset{\text{[acetic acid]}}{C_2H_4O_2} + CO_2 + H_2O$$

$$\underset{\text{[acetic acid]}}{C_2H_4O_2} + O_3 = \underset{\text{[formic acid]}}{CH_2O_2} + CO_2 + H_2O$$

$$\underset{\text{[formic acid]}}{CH_2O_2} + O = CO_2 + H_2O$$

Lecithin.—This substance ($C_{42}H_{84}NPO_9$) is a wax-like material, which can be separated from the nervous tissues and blood corpuscles. According to some observers it occurs in nervous tissues in combination with a nitrogenous glucoside called cerebrin to form protagon.

When boiled with an acid or alkali, lecithin yields glycero-phosphoric acid, stearic acid, and an alkaloid called neurine or choline.

Lecithin is abundantly found also in the seeds of many plants.[1]

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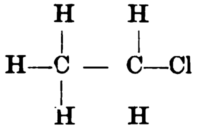
[1] Schulze and Steiger, *Zeit. physiol. Chem.* xiii. 365.

AROMATIC COMPOUNDS

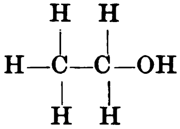
We have already seen how several different substances may exist, having the same formula. Isomerism, as this is termed, is due to differences in the atomic constitution of the molecules.

Take the substance known as ethyl chloride: this has the formula C_2H_5Cl, and can be represented graphically only under one possible form :—



$$\begin{array}{ccccc} & H & & H & \\ & | & & | & \\ H- & C & - & C & -Cl \\ & | & & & \\ & H & & H & \end{array}$$

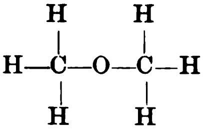
But in the case of a substance having the formula C_2H_5O, there are two possible arrangements of atoms :—



$$\begin{array}{ccccc} & H & & H & \\ & | & & | & \\ H- & C & - & C & -OH \\ & | & & | & \\ & H & & H & \end{array}$$

[ethyl alcohol]

and

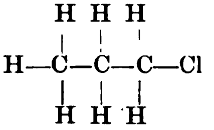


$$\begin{array}{ccccccc} & H & & & & H & \\ & | & & & & | & \\ H- & C & - & O & - & C & -H \\ & | & & & & | & \\ & H & & & & H & \end{array}$$

[methyl ether]

and as a matter of fact the two substances, ethyl alcohol and methyl ether, actually exist.

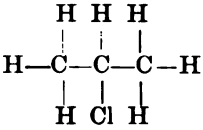
Or take the case of propyl chloride: again we have two, and only two, possible arrangements of atoms, and actually the two isomerides have been found to exist.



$$\begin{array}{ccccccc} & H & & H & & H & \\ & | & & | & & | & \\ H- & C & - & C & - & C & -Cl \\ & | & & | & & | & \\ & H & & H & & H & \end{array}$$

[orthopropyl chloride]

and



$$\begin{array}{ccccccc} & H & & H & & H & \\ & | & & | & & | & \\ H- & C & - & C & - & C & -H \\ & | & & | & & | & \\ & H & & Cl & & H & \end{array}$$

[isopropyl chloride]

In the case of compounds containing more than three atoms of carbon, the isomerides possible are more numerous, and those that actually exist are also more numerous. It does not, however, necessarily follow that actual compounds exist, corresponding to all the possible combinations, and a *law of limitation* is still wanting (Dittmar).[1]

One of the most striking of these instances is that of benzene ; this substance has the formula C_6H_6 ; there are more than thirty possible arrangements in which the atoms might be strung together, and yet only one benzene exists. Kekule[1] represents the constitution of benzene thus :—

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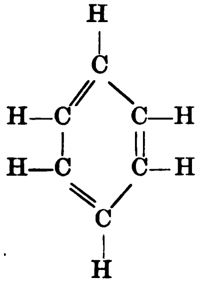
[1] McKendrick's *Physiology*, vol. i. p. 51.



that is, the six atoms of carbon do not form an open chain as in the substances of which the graphic formulæ have just been given, but a closed ring (the benzene ring). In this ring every two neighbouring C's are united alternately by a single and a double bond, and the fourth combining power of each C is satisfied by the H.

This substance benzene is the foundation of the aromatic group, which contains very numerous members. These can be derived by substituting one or more atoms of hydrogen, by more or less complicated radicles. If for instance one atom of hydrogen be replaced by one of chlorine, we obtain chlor-benzene, a substance of great stability. If one atom of hydrogen be replaced by hydroxyl (HO), an alcohol-like substance, *phenol* is obtained ; but it is distinguished from the alcohols in the same way that chlor-benzene is distinguished from the alcoholic chlorides : viz. the OH is more strongly attached than it is in the alcohols.

One of the hydrogen atoms is also replaceable by NO_2 (the radicle of nitric acid), to form nitro-benzene ($C_6H_5NO_2$), or by amidogen NH_2 (to form amido-benzene or aniline, $C_6H_5NH_2$), and thus nitrogenous aromatic compounds are obtained. Or, again, hydrogen may be replaced by radicles containing carbon, and so substances containing more than 6 atoms of carbon are added to the group ; for instance 1, 2, 3 or more atoms of the hydrogen are replaceable by methyl, and we get the following series :—

Benzene	C_6H_6
Methyl-benzene (Toluene) . .	$C_6H_5(CH_3)$
Di-methyl-benzene . . .	$C_6H_4(CH_3)_2$
Tri-methyl-benzene . . .	$C_6H_3(CH_3)_3$
Tetra-methyl-benzene . . .	$C_6H_2(CH_3)_4$

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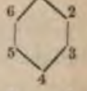
[1] Liebig's *Annalen*, vol. cxxxvii. p. 160.

Again a similar series is obtainable with ethyl, and all the other alcohol radicles; still further complication is produced by replacing some H's with one, and some with another kind, of radicle; and even more complicated compounds than these can be reached, because the atoms of hydrogen in the methyl, ethyl, &c., are replaceable by other elements, or other radicles.

In addition to all this, isomerism has to be reckoned with. If we represent the molecule of benzene by a hexagon, at the corners of which the carbon atoms are placed, it is seen that three isomeric di-methyl-benzenes can exist; in which the two methyl groups have the positions indicated by the figures:

a. 1 and 2. Ortho-di-methyl-benzene.
b. 1 and 3. Meta-di-methyl-benzene.
c. 1 and 4. Para-di-methyl-benzene.



No other cases of isomerism are here possible; as 1 and 6 is identical with 1 and 2; and 1 and 5 with 1 and 3.

One more example of this kind of isomerism may be given: pyrocatechin or catechol has the formula $C_6H_4\left\{\begin{matrix}OH\\OH\end{matrix}\right.$; i.e. two atoms of hydrogen in benzene are replaced by hydroxyl. This substance stands to phenol, as ethene glycol does to ethyl-alcohol; it is a diatomic phenol. But the hydroxyls may have different positions, as the methyls in the example just given, and thus we have two isomerides of pyro-catechin, which are known as resorcin and hydroquinone respectively.

We may now take *seriatim* the members of this important family, which are interesting to the physiologist, either because they occur in the body, or are useful as drugs or reagents.

Phenol or Carbolic acid $C_6H_5.OH$ is a white crystalline substance, fusing at 42°C., boiling at 184°, and forming the chief constituent of the heavy coal oils. Perchloride of iron gives with it a deep violet colour. A chip of fir or deal moistened with phenol and then with dilute hydrochloric acid and exposed to the light turns a deep greenish blue. Phenol reduces silver nitrate. When heated with nitric acid, tri-nitrophenol $C_6H_2(NO_2)_3OH$, commonly called picric acid, is formed. An aqueous solution of phenol gives with bromine water a yellowish crystalline precipitate of tri-bromo-phenol ($C_6H_2Br_3.OH$). This reaction may be used for the quantitative determination of phenol. Phenol gives in addition the following colour reactions:

a. A blue or greenish colour, on adding a quarter of its volume of ammonia and a few drops of potassium chloride.

b. A blue colour in presence of a little aniline and an alkaline solution of sodium hypochlorite.

c. An intense red colour with Millon's reagent.

d. A brown colour changing to green and blue on adding fuming nitric acid, or a 6 per cent. solution of potassium nitrite in strong sulphuric acid.

Phenol occurs normally in the urine, sweat, and fæces in small quantities, but especially after medical or surgical treatment with carbolic acid or other drugs containing a benzene nucleus. It is seldom present in the free state, but usually as phenol sulphate of potassium ($C_6H_5O.SO_3K$). The dark colour of the urine in carboluria is due to one or both of the two isomerides, pyrocatechin and hydrochinon. On exposure to the air (oxidation) in an alkaline urine these substances turn dark brown. Phenol is formed by the activity of the pancreatic ferment and putrefactive organisms on proteids in the intestine. That normally in the urine is absorbed from the intestine.

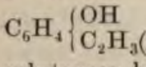
Pyrocatechin or Catechol $C_6H_4(OH)_2$.—This occurs in small quantities as a conjugated-sulphate in the urine. It is a crystalline substance, which turns brown on oxidation in alkaline solutions, and green on admixture with ferric chloride. It was called alcapton by Bodeker, when he found it in abnormally large quantities in certain urines. It must be carefully distinguished from sugar, as it reduces alkaline solutions of copper salts like Fehling's solution. It occurs in the cerebro-spinal fluid. It is one of the products of the decomposition of proteids.

Cresol $C_7H_7(HO)$.—This is a derivative of toluene or methylbenzene (C_7H_8). It is contained in crude carbolic acid. It boils at 200°C. It also is a product of the decomposition of proteids, and so is found in the fæces, and a small quantity passes into the urine as cresol sulphate of potassium.

Benzyl alcohol has the formula $\left.\begin{matrix}C_6H_5CH_2\\H\end{matrix}\right\}O$; its aldehyde is $C_6H_5.COH$, i.e. the alcohol *minus* H_2; and an acid is formed by replacing the H_2 by O. The aldehyde is known as oil of bitter almonds, and is the result of the decomposition of the amygdalin contained in the almond. The acid is called *benzoic acid* $C_7H_6O_2$. This occurs in the urine, especially of herbivora, combined with glycocine or amido-acetic acid ($C_2H_3O_2.NH_2$) to form *hippuric acid* ($C_9H_9NO_3$). The radicle of benzoic acid is called *benzoyl* (C_7H_5O).

Salicylic or Oxybenzyl group.—The members of this group are closely connected with the benzoyl group; they are benzoyl compounds in which an atom of hydrogen is replaced by hydroxyl (HO).

The formula for *salicylic acid* is $C_7H_6O_3$. If two atoms of hydrogen in this be replaced by hydroxyl, a substance is obtained with the formula $C_7H_6O_5$, which is called *gallic acid.* This acid is generally obtained from nut-galls. On heating it splits up into CO_2 and *pyrogallic acid* (tri-hydroxyl-benzene $C_6H_3(OH)_3$).

Tri-methyl-benzene $C_6H_3(CH_3)_3$ is the starting-point of the aromatic compounds containing nine atoms of carbon. The most important members of the group are *anethol*, the chief constituent of anise oil; *anisamic acid*, which exists in the balsams of Tolu and Peru; *cumarin*, found in Tonka-bean, and sweet scented grasses; *tyrosine*  $C_6H_4\left\{\begin{array}{l}OH\\C_2H_3(NH_2)CO_2H\end{array}\right.$, a product of the decomposition of albuminous substances, hair, horn, &c.; it is found also in the cochineal insect. Being an amido-acid, it will be more fully described with that group.

Thymol, an important antiseptic, contained in oil of thyme, is a derivative of tetra-methyl-benzene.

Aromatic oxy-acids.—Two of these, hydroparacumaric acid and paraoxyphenyl acetic acid, are found in the urine in combination with potassium in small quantities. They apparently are derived from the decomposition that takes place in proteids in the intestine; tyrosine is probably an intermediate product (Baumann).[1]

$$\underset{\text{[tyrosine]}}{C_9H_{11}NO_3}+H_2=\underset{\text{[hydroparacumaric acid]}}{C_9H_{10}O_3}+NH_3$$

$$\underset{\text{[hydroparacumaric acid]}}{C_9H_{10}O_3}=\underset{\text{[paraethyl phenol]}}{C_8H_{10}O}+CO_2$$

$$\underset{\text{[paraethyl phenol]}}{C_8H_{10}O}+O_3=\underset{\text{[paraoxyphenyl acetic acid]}}{C_8H_8O_3}+H_2O$$

The Indigo Group.—Substances belonging to this group are found not only in the vegetable kingdom, but also in animals. The pure colouring matter obtained from the crude commercial product is called *indigotin or indigo-blue* C_8H_5NO. This by the action of reducing agents becomes *indigogen or indigo-white*, C_8H_6NO. On oxidation a body called *isatin* is formed, $C_8H_5NO_2$. When indigo-white is heated with zinc and water it yields *indole* C_8H_7N; and when isatin is acted on by potash, it yields aniline ($\underset{\text{[isatin]}}{C_8H_5NO_2}+4KOH=\underset{\text{[aniline]}}{C_6H_7N}+2K_2CO_3+H_2$).

This last reaction shows that the indigo group of substances contains the benzene group of atoms.

The parent of all these substances in plants is a colourless substance called *indican* $C_{26}H_{31}NO_{17}$. Indican is a glucoside; when boiled with acids, it splits into indigo-blue, and a sugar-like substance called indiglucin. $\underset{\text{[indican]}}{C_{26}H_{31}NO_{17}}+2H_2O=\underset{\text{[indigo-blue]}}{C_8H_5NO}+\underset{\text{[indiglucin]}}{3(C_6H_{10}O_6)}$.

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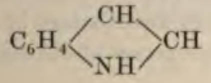
[1] *Zeit. physiol. Chem.* vol. x. p. 123.

The starting-point of the indigo series from a chemical point of view is indole. We pass from indole to indigo-blue by successive oxidations, and from indigo-blue to indole by successive reductions. A full list of the various intermediate products with their formulæ is as follows:—

Indole C_8H_7N
Ox-indole C_8H_7NO
Diox-indole $C_8H_7NO_2$
Isatyde $C_8H_6NO_2$

Isatin $C_8H_5NO_2$
Indigo-white C_8H_6NO
Indigo-blue C_8H_5NO

The formula for indole is thus represented graphically by Baeyer and Emmerling:—



Of this group, two members are found in the body, viz. indole (from which indigo is obtainable) and skatole, a derivative of indole.

Indole C_8H_7N is an oily fluid, which crystallises when mixed with water; the crystals melt at 52°C. It has a fæcal odour and is readily soluble in alcohol and ether. It gives a red precipitate with dilute fuming nitric acid. This precipitate is soluble in alcohol, and the alcoholic solution, mixed with hydrochloric acid, colours fir-wood cherry red, changing after a while to dirty brown red (Baeyer).

It is a product of the decomposition of proteids, and is formed from these substances during their stay in the intestine. It passes away partly with the fæces; part is absorbed, and finally excreted with the urine as an ethereal sulphate.

Indigo.—If the urine (especially in diseases where much putrefaction occurs in the alimentary canal, or after the administration of certain drugs—creosote, benzoic aldehyde, turpentine, &c.) be boiled with a large quantity of strong hydrochloric and a few drops of nitric acid, a violet-red colour appears, due to the formation of indigo-blue and indigo-red. There are also other methods employed for demonstrating the fact that, on oxidation, indigo may be obtained from the urine. The parent substance in the urine is called *indican*, but this must not be confounded with the indican of vegetables. Vegetable indican is a glucoside. The indican of urine is indoxyl-sulphate of potassium, and is derived from the indole of the intestine. Its formula is $C_8H_6NSO_4K$ (i.e. the radicle of potassium sulphate, KSO_3, *plus* indoxyl, C_8H_6NO). This substance does not apparently occur in the sweat, or only in traces; its presence has, however, been stated to have been demonstrated in cases of *chromidrosis*, or coloured sweat (Bizio, Hofmann).

Skatole C_9H_9N is methyl indole, $C_8H_6(CH_3)N$, i.e. indole in which an H is replaced by methyl, CH_3. When pure it occurs in dentate shining plates, having a fæcal odour, and melting at 94°C. Fuming nitric acid gives with its solutions a white, cloudy precipitate, thus distinguishing it from indole. From its hydrochloric acid solution, it is thrown down, on the addition of picric acid, in the form of red needles. When present in the urine, it gives a violet-red colour with strong hydrochloric acid and chloride of lime.

Skatole like indole is formed in the alimentary canal from proteids;[1] most is excreted *per rectum*; a small quantity is absorbed, and finally excreted in the urine and sweat. Absorption of a large quantity of indole, skatole, &c., produces disturbances of the nervous system; and many of the unpleasant symptoms of constipation may arise from this cause.

In the urine skatole is found, like indole, in the form of an ethereal sulphate. The name of this compound is skatoxyl sulphate of potassium ($C_9H_8NSO_4K$). It has been surmised that this substance may, like the corresponding indoxyl compound, give rise to a pigment. Mester[2] finds, however, that the amount of the so-called skatole-pigment is not proportional to the amount of the skatoxyl sulphuric acid; and he suggests that the chromogen of the pigment is a combination of skatoxyl with glycuronic acid.

Skatoxyl-potassium-sulphate is also present in the sweat (Kast).[3]

NITROGENOUS ORGANIC COMPOUNDS

A few nitrogenous organic compounds have already been described; we have to deal now more especially with the organic derivatives of ammonia.

Amines.—An amine is a compound ammonia, which can be obtained by replacing one or more atoms of the hydrogen in ammonia (NH_3) by alcohol radicles. Of these only one has been described in the body—*trimethylamine* $N(CH_3)_3$, which occurs normally in human urine, and is found in guano, decomposing fish, and decomposing proteids generally. It is the substance to which the characteristic smell of fish is due. It is an oily fluid, strongly alkaline, soluble in alcohol, ether, and water. It boils at 9° C.

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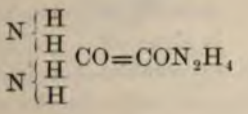
[1] First found there by Brieger (*Ber. d. deutsch. chem. Ges.* vol. viii. p. 722), and Nencki (*Centralbl. Med. Wiss.* 1878, No. 47).

[2] *Zeit. physiol. Chem.* xii. 130.

[3] *Ibid.* xi. 501.

Naphthylamine ($C_{10}H_9N$) is a product of the oxidation of proteids, and naphthalene ($C_{10}H_8$) has been detected by Hoppe-Seyler in the urine.

Amides are derivatives of acids which have exchanged the hydroxyl (HO) of the acid for amidogen (NH_2). *Urea* (CON_2H_4) is a typical member of the group. By some it is regarded as the diamide of carbonic acid. Hydrogen carbonate has the formula $CO(OH)_2$; replace the hydroxyls by amidogen, and we get $CO(NH_2)_2$. From another point of view it may be regarded as being built in the type of two molecules of ammonia, in which two hydrogen atoms are replaced by the dyad radicle CO :—



$$\begin{matrix} N\left\{\begin{matrix} H \\ H \end{matrix}\right. \\ N\left\{\begin{matrix} H \\ H \end{matrix}\right. \end{matrix} CO = CON_2H_4$$

It is thus carbamide.

Urea is isomeric with ammonium cyanate $(NH_4)CNO$, from which it was first prepared synthetically by Wöhler (1828). When ammonium cyanate is heated to 100°C., the atoms rearrange themselves to form urea. It may also be prepared by the action of ammonia on carbonyl chloride ($COCl_2 + 4NH_3 = CON_2H_4 + 2NH_4Cl$).

By uniting with water, urea forms ammonium carbonate. This it does under the influence of a specific organised ferment (*micrococcus ureæ*) in decomposing urine ($CON_2H_4 + 2H_2O = (NH_4)_2CO_3$).

Urea is met with in nearly all the solids and fluids of the body, but chiefly in the urine; about 30 grammes (500 grains) are on the average excreted by the kidneys of an adult daily. Urea is the chief end product of the metabolism of the nitrogenous constituents of the body.

Oxaluric acid is urea in which one atom of hydrogen is replaced by the radicle of oxalic acid (i.e. oxalic acid *minus* HO).

The Amido-Acids are compounds which show partly the character of an acid, and partly that of a weak base. They may be considered as ammonias, in which one or more atoms of hydrogen are replaced by radicles of an acid, thus resembling alkaloids; or, on the other hand, as acids in which one or more hydrogen atoms of the acid radicle are replaced by amidogen (NH_2). The principal are as follows :—

(*a*) *Glycocine.*—This is also known as glycine, glycocoll, and amido-acetic acid, the last-mentioned name expressing its constitution. The formula for acetic acid is $C_2H_4O_2$; if one atom of hydrogen be replaced by NH_2, we obtain $C_2H_3(NH_2)O_2=C_2H_5NO_2$, which is the formula for glycocine or amido-acetic acid. It has been obtained synthetically by the action of monochloracetic acid on ammonia ($C_2H_3ClO_2+NH_3=HCl+C_2H_3(NH_2)O_2$). In a pure state glycocine crystallises in rhombohedric prisms, soluble in water, but not in ether or alcohol. The aqueous solution is acid.

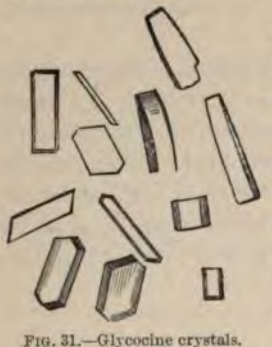


FIG. 31.—Glycocine crystals.

When heated on platinum foil, it leaves a colourless residue, which, on warming with a drop of caustic soda solution, forms an oily drop which runs about without touching the surface of the platinum. (Scherer's test.) When heated in a glass tube open at both ends, it sublimes, and a smell of amylamine is given off.

Glycocine is found in combination in glycocholic acid (one of the bile acids), and in hippuric acid (in the urine). It is a product of decomposition of proteids, and also of gelatin, mucin, and other albuminoids. It occurs free in small quantities in the intestine as a result of the decomposition of the bile that occurs there. It is probably largely reabsorbed as such. Part may be transformed into urea.

(*b*) *Leucine* ($C_6H_{13}NO_2$) is amido-caproic acid, i.e. caproic acid ($C_6H_{12}O_2$) in which an H is replaced by NH_2: or, according to some, oxy-caproic or leucic acid ($C_6H_{12}O_3$), in which hydroxyl (HO) is replaced by NH_2.

Leucine forms yellowish-brown spheres consisting of masses of needle-shaped crystals, soluble in water and slightly soluble in alcohol, but insoluble in ether. By heat it is decomposed into carbonic acid and amylamine ($C_6H_{13}NO_2=CO_2+C_5H_{11}.NH_2$); by hydriodic acid into caproic acid and ammonia ($C_6H_{13}NO_2+H_2=C_6H_{12}O_2+NH_3$); with sulphuric acid it yields ammonia and valerianic acid; with potassium permanganate, oxalic acid, carbonic acid, valerianic acid and ammonia. Probably similar decompositions occur in the body. Probably also leucine is one of the intermediate products in the formation of urea.

Leucine is most important as one of the chief decomposition products of proteids, and is formed when proteids are decomposed with strong acids or alkalis, or undergo putrefaction, and within the body by the activity of certain ferments, especially of one secreted by the pancreas called trypsin. It is found in small quantities as a constituent of many organs and tissues, particularly of the pancreas. In certain cases, however (spleen, thymus, &c.), it appears to be formed by *post-mortem* changes, and not to be a constituent of the healthy living tis may be separated from an organ by making a watery extract; this is boiled, acidified and filtered to separate any proteid; to the filtrate basic lead acetate is added, and again it is filtered; excess of lead is removed from the filtrate by a stream of sulphuretted hydrogen. The lead sulphide is filtered off, the filtrate is evaporated to a syrup, and extracted with hot alcohol. The alcoholic extract is evaporated, and the residue is leucine.

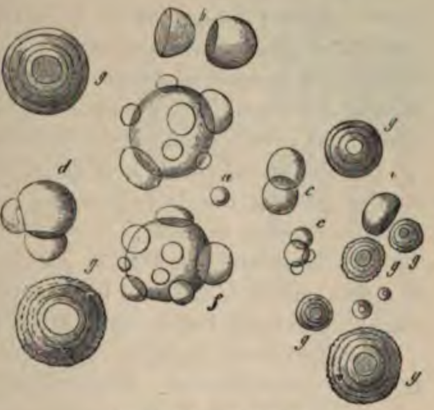


FIG. 32.—Leucine crystals.

(c) *Tyrosine* $C_9H_{11}NO_3$ is amido-oxy-phenyl-propionic acid.

Propionic acid has the formula $C_3H_6O_2$. Amido-propionic acid $C_3H_5(NH_2)O_2$ is also called *alanine*. Oxyphenyl-propionic acid is propionic acid in which one H is replaced by oxyphenyl ($C_6H_4.OH$), i.e. $C_3H_5(C_6H_4.OH)O_2$; if another H in this be replaced by NH_2 we get $C_3H_4(NH_2)(C_6H_4.OH)O_2=C_9H_{11}NO_3$, which is amido-oxyphenyl-propionic acid or tyrosine.

To separate tyrosine from an albuminous fluid, the fluid must be boiled and the precipitated proteid filtered off. The filtrate is evaporated to a third of its volume on the water-bath, precipitated with lead acetate and filtered, a stream of sulphuretted hydrogen is passed through the filtrate, and the lead sulphide so precipitated, filtered off; the filtrate is evaporated, and crystals of tyrosine separate out.

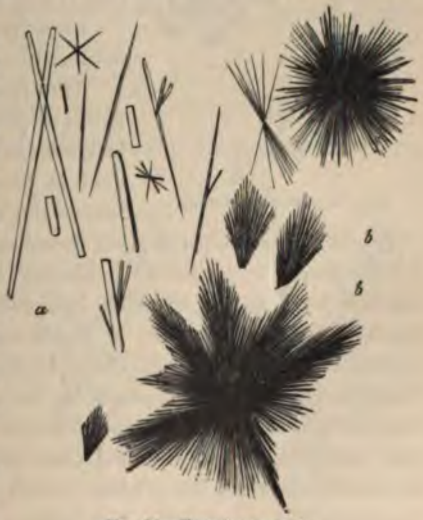


FIG. 33.—Tyrosine crystals.

Tyrosine crystallises in slender needles, often in groups, slightly soluble in water, but insoluble in alcohol and ether. On oxidation it yields benzoic aldehyde, hydrocyanic, benzoic, acetic, formic, and carbonic acids.

Tests. i. Heat in a watch-glass with concentrated sulphuric acid; cool; add water and a few pieces of chalk; there will be an effervescence; filter; evaporate to a small bulk; add a few drops of a neutral solution of ferric chloride; a violet colour is produced (Piria).[1] ii. Millon's reagent gives a red solution, the tint of which deepens on heating (Hoffmann).[2] Tyrosine is generally found along with leucine, and like it results from the decomposition of proteids.

(*d*) *Amido-valerianic acid* ($C_5H_{11}NO_2$) is a product of decomposition of proteids, but it only occurs in small quantities in comparison with leucine and tyrosine.

(*e*) *Sarcosine* $C_3H_7NO_2$ is methyl-glycocine, i.e. amidoacetic acid in which one H is replaced by methyl $C_2H_2(CH_3)(NH_2)O_2$. It is not found in the body, but is a product of decomposition of creatine.

(*f*) *Creatine* $C_4H_9N_3O_2$. Sarcosine ($C_3H_7NO_2$) united to cyanamide $CN.NH_2$ gives creatine. When creatine is boiled with baryta water, it takes up water and yields sarcosine and urea

$$(\underset{\text{[creatine]}}{C_4H_9N_3O_2}+H_2O=\underset{\text{[sarcosine]}}{C_3H_7NO_2}+\underset{\text{[urea]}}{CON_2H_4}).$$

Creatine crystallises with one molecule of water of crystallisation in colourless rhombohedric prisms, soluble in water, almost insoluble in alcohol. The aqueous solution is neutral. On oxidation it yields methyl-uramine ($C_2H_7N_3$), oxalic acid and carbonic acid.

Creatine has been found in the muscles, nerves, blood, liquor amnii and testis.

The question as to whether it is an intermediate product in the formation of urea is unsettled (*see* muscle). No doubt a good deal is transformed into creatinine, which leaves the body by the urine.

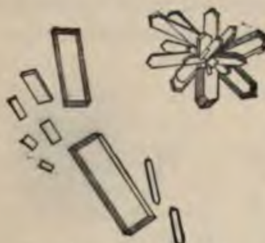


FIG. 34.—Creatine crystals.

(*g*) *Creatinine* $C_4H_7N_3O$ is creatine *minus* H_2O. It can be formed by heating the latter with boiling water for a long time; or more readily by heating it with hydrochloric acid. When creatinine is heated with baryta water it yields methylhydantoin ($C_4H_6N_2O_2$) and ammonia ($C_4H_7N_3O+H_2O=C_4H_6N_2O_2+NH_3$). It crystallises in large colourless prisms soluble in water and alcohol, but almost insoluble in ether. It has an alkaline taste and reaction. Salkowski[3] has, however, recently stated that the greater part of this alkalinity is due to adherent impurities.—*Tests.* i. With zinc-chloride it gives a characteristic crystalline precipitate (groups of fine needles). This consists of a combination of zinc-chloride with creatinine ($C_4H_7N_3O.ZnCl_2$). This test is used for the quantitative estimation of creatinine. ii. A solution of creatinine, as in urine, acidulated by nitric acid gives with phosphomolybdic acid a yellow crystalline precipitate soluble in hot nitric acid. iii. It reduces an alkaline solution of cupric hydrate such as Fehling's solution.

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[1] *Ann. Chem. Pharm.* vol. lxxxii. p. 252.

[2] *Ibid.* vol. lxxxvii. p. 123. *See* also L. Meyer, *ibid.* vol. cxxxii. p. 156.

[3] *Zeit. physiol. Chem.* xii. 211.

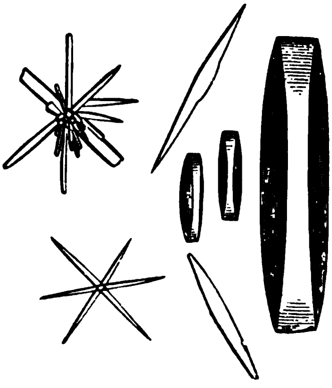
FIG. 35.—Creatinine crystals.

(*h*) *Taurine* $C_2H_7NSO_3$ is amido-isethionic acid. Isethionic acid is sulphurous acid in which an atom of hydrogen is replaced by the monatomic radicle oxy-ethylene ($C_2H_4.OH$). If the hydroxyl of this radicle be replaced by NH_2 we obtain taurine. The following formulæ will assist in the understanding of this relationship :

H_2SO_3, Sulphurous acid.
$H(C_2H_4.OH)SO_3$, Isethionic acid.
$H(C_2H_4.NH_2)SO_3=C_2H_7NSO_3$, Taurine.

Taurine is artificially prepared by heating ammonium isethionate ($C_2H_9NSO_4$) which parts with H_2O and so yields taurine.

Cyanic acid with taurine forms tauro-carbamic acid, in which form taurine is partially excreted in the urine

$$(\underset{\text{[cyanic acid]}}{CNHO}+\underset{\text{[taurine]}}{C_2H_7NSO_3}=\underset{\text{[tauro-carbamic acid]}}{C_3H_8N_2SO_4}.$$

Taurine is probably partially disintegrated into simpler substances, and it has been conjectured that the alkalis of the bile may act upon it, and thus give rise to some of the sulphates in the urine. Taurine crystallises in colourless six-sided prisms, soluble in water, but insoluble in alcohol and in ether.

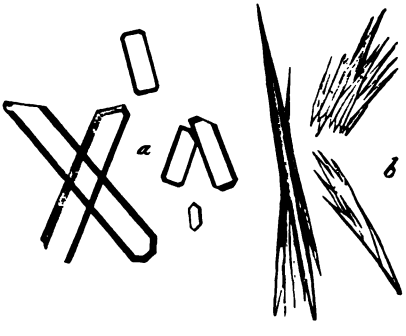

FIG. 36.—Taurine crystals. *a*, pure ; *b*, impure.

It occurs in the body in combination as tauro-cholic acid in the bile. A small quantity of it occurs free in the fæces from the decomposition of this acid that occurs in the intestine ; the greater amount that leaves the body, passes into the urine in the manner just mentioned. Probably the greater quantity of the taurine formed in the intestine is reabsorbed as such. Small quantities of taurine have been separated from liver, muscle, urine (of ox), and spleen (of certain fishes).

(*i*) *Cystine* $C_3H_7NSO_3$ is amido-lactic acid in which one H is replaced by HS.

Lactic acid :—$C_3H_6O_3$.
Amido-lactic acid :—$C_3H_5(NH_2)O_3$.
Cystine :—$C_3H_4(HS)(NH_2)O_3=C_3H_7NSO_3$.

If heated on a silver surface it gives a black spot of silver sulphide.

It crystallises in the hexagonal system either as colourless hexagonal plates or rhombohedra. The crystals are insoluble in water, alcohol, and ether, but soluble in alkalis, mineral acids, and oxalic acid.

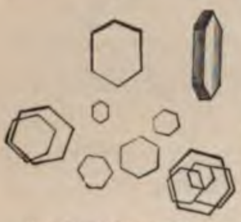

FIG. 37.—Cystin crystals.

It is found as a constituent of a rare form of urinary calculus. Its origin is unknown, but cystinuria appears to be hereditary. On exposure to the air these calculi turn green ; they form only in acid urine owing to their solubility in alkaline fluids.

According to recent observations by Goldmann and Baumann,[1] cystine is a normal constituent of urine, but is present in very minute quantities.[2]

(*j*) *Aspartic* (*or asparaginic*) *acid* $C_4H_7NO_4$ is amido-succinic acid. It is obtained from the substance asparagine ($C_4H_8N_2O_3$) in plants ; and is a product of the decomposition of proteids.

(*k*) *Glutamic* (*or glutaminic*) *acid* $C_5H_9NO_4$ is amido-glutaric acid, i.e. an amido-compound of the same series to which succinic acid belongs (the oxalic acid series, *see* p. 67). This is also a product of the decomposition of proteids.

(*l*) *Carbamic acid* CH_3NO_2 is amido-formic acid. It is not known in the free state ; its ammonium salt is a product of the decomposition of proteids.

The Bile Acids.—The bile contains the sodium salts of complex amido-acids called the bile acids. The two acids found in human bile are glycocholic acid and taurocholic acid.

Glycocholic acid $C_{26}H_{43}NO_6$ is especially abundant in the bile of herbivora, and in man its amount is increased by a vegetable diet. By the action of dilute acids or alkalis and also in the intestine it takes up water and splits into glycocine and cholalic acid.

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[1] *Zeit. physiol. Chem.* xii. 254.

[2] Delépine (*Proc. Roy. Soc.* vol. xlvii. 1890, p. 198) states that the formation on cystine may result from the activity of a torula-like organism in the urine.

$$\underset{\text{[glycocholic acid]}}{C_{26}H_{43}NO_6} + H_2O = \underset{\text{[glycocine]}}{C_2H_5NO_2} + \underset{\text{[cholalic acid]}}{C_{24}H_{40}O_5}$$

It forms brilliant colourless needles soluble in water and alcohol, but not in ether. Its taste is first sweet and afterwards bitter. The alcoholic solution is dextrorotatory $(\alpha)_D = +29°$.

The glycocholate of soda $C_{26}H_{42}NaNO_6$ is the compound that occurs in the bile. It crystallises in stellate needles, soluble in water and alcohol, but not in ether. $(\alpha)_D = +25·7°$. *Glycocholate of potash* occurs in the bile of certain fishes.

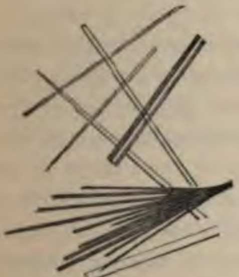

FIG. 38.—Sodium Glycocholate.

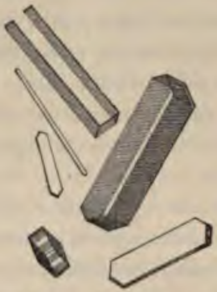

FIG. 39.—Cholalic Acid.

Taurocholic acid $C_{26}H_{45}NO_7S$ is especially abundant in the bile of carnivora. By the action of hydrating reagents and in the intestine it is decomposed into taurine and cholalic acid.

$$\underset{\text{[taurocholic acid]}}{C_{26}H_{45}NO_7S} + H_2O = \underset{\text{[cholalic acid]}}{C_{24}H_{40}O_5} + \underset{\text{[taurine]}}{C_2H_7NO_3S}.$$

It forms silky needles, soluble in water and alcohol, and intensely bitter. $(\alpha)_D = +24·5°$. *Sodium taurocholate* $C_{26}H_{44}NaNO_7S$ is the compound that occurs in the bile, except in certain fishes where the potassium salt is found.

Cholalic (or cholic) acid $C_{24}H_{40}O_5$ derived from the bile acids, forms large, shining, deliquescent crystals, slightly soluble in water, and readily soluble in alcohol and ether. $(\alpha)_D = +35°$. Boiled with acids or heated to 200°, it loses either one molecule of water to form choloidic acid, or two to form dyslysin ($C_{24}H_{36}O_3$). Latschinoff has recently assigned to cholalic acid the formula $C_{25}H_{42}O_5$, but Mylius[1] has shown that the correct formula is without doubt that originally assigned to it by Strecker $C_{24}H_{40}O_5$.

Choleic acid is an acid which has been separated from ox-bile by

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[1] *Ber. d. deutsch. chem. Gesell.* xix. 369, 2000; xx. 1968.

Latschinoff.[1] It occurs in two forms: anhydrous $C_{25}H_{42}O_4$, and hydrated $C_{25}H_{42}O_4 + 1\frac{1}{2}H_2O$. The latter is called deoxy-cholic acid by Mylius.[2]

Fellic acid.—The cholalic acid from human bile differs in some of its reactions and solubilities from that obtained from ox-bile (Hammarsten).[3] Bäyer[4] calls it anthropo-cholalic acid and assigns to it the formula $C_{18}H_{28}O_4$. Schotten[5] suggested that the difference was due to an admixture with choleic acid. He, however, subsequently found this was not the case, but that another acid is present with formula $C_{23}H_{40}O_4$, to which the name fellic acid is given. It is due to admixture with fellic acid that cholalic acid from human bile appears to be different from that obtained from other sources.

Hyo-glycocholic and hyo-taurocholic acids combined with soda form the bile salts of pig's bile. In these acids cholalic acid is replaced by a nearly related acid called hyo-cholalic acid ($C_{25}H_{40}O_5$). The hyo-glycocholate is more abundant than the hyo-taurocholate, and has been separated by Jolin[6] into two varieties a and β, the former of which is precipitable by sodium sulphate, the latter not. In the bile of the goose cholalic acid is replaced by *cheno-cholalic* acid ($C_{27}H_{44}O_4$).

Pettenkofer's Reaction.—If a thin film of bile be spread on a porcelain dish, a drop of solution of cane sugar, and a drop of strong sulphuric acid be added, a beautiful purple colour is developed, especially on the application of heat. This test is given by all the acids and salts found in the bile. The reaction is due to the formation of furfuraldehyde from the sugar and sulphuric acid; the furfuraldehyde forms a coloured compound with cholalic acid. It is by no means distinctive of bile acids, however, as Mylius[7] and Udranszky[8] have shown. Of the numerous organic substances which give the colour or a very similar one, one only, a-naphthol, gives it more readily than the bile acids. The spectroscopic appearances differ, however, in many instances. In the case of the colour produced by bile there is a band between D and E and another at F.

The Uric Acid Group.—(*a*) *Uric acid* $C_5H_4N_4O_3$.—There is much diversity of opinion with regard to the chemical nature of this substance; the different views held on this point, and also its relationship to urea, and to other substances of a similar nature (allantoin, alloxan, xanthine, &c.) will be more appropriately discussed in connection with the physiological uses of these bodies (*see* Urine).

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[1] *Ber. d. deutsch. chem. Gesell.* xviii. 3039.

[2] *Ibid.* xix. 369.

[3] *Maly's Jahresb.* 1878, p. 263.

[4] *Zeit. physiol. Chem.* ii. 358; iii. 292.

[5] *Ibid.* xi. 268.

[6] *Ibid.* xi. 417.

[7] *Ibid.* xi. 492.

[8] *Ibid.* xii. 355. Udranszky here gives a list of 76 organic substances that give the furfuraldehyde reaction.

Pure uric acid crystallises in colourless rhombic plates or prisms. When obtained from urine it is more or less tinged with pigment, and it assumes many crystalline forms (dumb-bells, whetstones, &c.). It is without taste or smell. It is insoluble in alcohol and in ether; it requires for its solution 15,000 parts of cold and 1,900 of hot water. Its solutions give only a feeble acid reaction.

It is not found in the free condition in the urine, except in cases of disease (gravel, calculus); but it is combined with bases to form urates.[1] The acid is dibasic.

The amount excreted per diem by an adult averages 0·5 to 1 gramme, but its amount is raised by much animal food, and by want of exercise. Urates occur also in the blood, and as chalky deposits in and around the cartilages of gouty persons. The solid urine of birds and reptiles consists almost entirely of urates. Traces of uric acid have been separated from various tissues, kidneys, spleen, lungs, brain and muscle.

Urates of sodium.—The neutral salt $C_5H_2N_4O_3Na_2$ forms nodular masses, and the acid salt $C_5H_3N_4O_3Na$ is usually amorphous in urine; they form the deposit in urine commonly called lithates. The chalky deposits in gout are chiefly composed of the acid salt, which is then crystalline.

Urates of potassium corresponding to these seldom occur.

Acid ammonium urate $C_5H_3N_4O_3(NH_4)$ (neutral salt unknown) forms globular collections of crystals; it is found in the deposit in alkaline urine, and is the chief component of the excrement of reptiles and birds.

Acid calcium urate $(C_5H_3N_4O_3)_2Ca$ occurs in the form of fine needles in urinary sediments, calculi, and in gouty deposits.

Acid lithium urate $C_5H_3N_4O_3Li$ is the most soluble salt of uric acid, hence the use of lithia as a drug in cases of gouty diathesis.

Murexide test.—Evaporate to dryness with nitric acid; the residue is reddish yellow, and becomes reddish purple on the addition of ammonia, and bluish violet with soda or potash.

(*b*) *Xanthine* $C_5H_4N_4O_2$ differs from uric acid by containing one atom of oxygen less. It is a pale yellow, amorphous powder, insoluble in alcohol or ether, soluble in cold water. When evaporated to dryness with nitric acid, a yellowish residue remains which turns red with caustic potash, and reddish violet on being heated.

Xanthine occurs normally in minute quantities in the urine, and has been obtained from many organs such as pancreas, spleen, liver, brain, and thymus.

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[1] The question of quadurates will be discussed under 'Urine.'

Urinary calculi, consisting of xanthine, varying in size from a pea to a pigeon's egg, occasionally form.

(*c*) *Hypoxanthine* $C_5H_4N_4O$ differs from xanthine by containing one atom of oxygen less. It generally occurs with xanthine. It has been described in the spleen, pancreas, muscles, liver, marrow, blood, and urine. In leucocythæmia its quantity in the blood and urine is increased. In acute yellow atrophy, the amount in the liver rises.

(*d*) *Adenine* $C_5H_5N_5$ can be obtained from the nuclei of cells. On heating it with sulphuric acid NH is replaced by O, and hypoxanthine thus formed : $\underset{\text{[adenine]}}{C_5H_5N_5} + H_2O = \underset{\text{[hypoxanthine]}}{C_5H_4N_4O} + NH_3$. Both substances contain a radicle $C_5H_4N_4$ called adenyl (Kossel[1]). Adenine is a crystalline substance ; the crystals contain three molecules of water of crystallisation.

(*e*) *Guanine* $C_5H_5N_5O$ has been found in the liver and pancreas, in guano, in the excrement of spiders, and in the skin of many reptiles and fishes. It bears the same relation to xanthine that adenine does to hypoxanthine ($\underset{\text{[guanine]}}{C_5H_5N_5O} + H_2O = \underset{\text{[xanthine]}}{C_5H_4N_4O_2} + NH_3$). It is amorphous, insoluble in water, alcohol, and ether, but readily soluble in acids and alkalis. The crystals of chlorate of guanine are characteristic.

(*f*) *Allantoin* $C_4H_6N_4O_3$ is found in the amniotic and allantoic fluids and in the urine of new-born animals. It crystallises in colourless prisms which are soluble in water. By the action of dilute nitric acid it takes up water and splits into allanturic acid ($C_7H_{10}N_6O_6$) and urea.

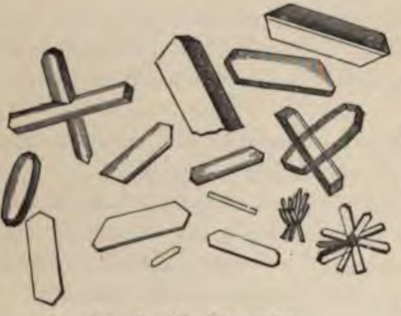

FIG. 40.—Allantoin crystals.

(*g*) *Carnine* $C_7H_8N_4O_3$ which has been separated from muscle, is a crystalline substance, the crystals containing one molecule of water of crystallisation. It is soluble in warm water, insoluble in alcohol and ether. It may be considered as a compound of hypoxanthine and acetic acid :

$$\underset{\text{[hypoxanthine]}}{C_5H_4N_4O} + \underset{\text{[acetic acid]}}{C_2H_4O_2} = \underset{\text{[carnine]}}{C_7H_8N_4O_3}$$

Other Nitrogenous Acids.—(*a*) *Inosinic or Inosic acid* $C_{10}H_{14}N_4O_{11}$.

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[1] *Zeit. physiol. Chem.* xiii. 292. Hypoxanthine is adenyl oxide; adenine is adenyl imide. Compounds of the radicle NH are called imides.

An uncrystallisable substance of doubtful nature, which has been described as a constituent of muscle juice.

(*b*) *Cryptophanic acid* $C_{10}H_{18}N_2O_{10}$ an amorphous acid said to exist in small quantities in human urine (Thudichum).[1]

(*c*) *Sulpho-cyanic acid* CNHS united to potassium or sodium to form a sulpho-cyanide (CNKS) is found in saliva, and occasionally also in urine, milk, and blood. It gives a red colour with ferric chloride, due to the formation of sulpho-cyanide of iron.

(*d*) *Cynurenic acid* $C_{20}H_{14}N_2O_6$ is found in dog's urine.[2] It is a decomposition product of proteids, but is apparently not derived from the putrefaction, which occurs in those substances in the alimentary canal (Baumann).[3] On heating its crystals, which contain two molecules of water of crystallisation, to 250° a base called cynurin $C_{18}H_{14}N_2O_2$ is obtained.[4] By means of certain reagents Kretschy[5] obtained chinolin C_9H_7N from it.

(*e*) *Urocanic acid* $C_6H_6N_2O_2+2H_2O$ was found in the urine of a dog in which the urea was diminished. At 212° C. it breaks up into carbonic acid, water, and a base urocanin $C_{11}H_{10}N_4O$ (Jaffe).[6]

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[1] *Journ. Chem. Soc.* (2) viii. 182.

[2] Hofmeister, *Zeit. physiol. Chem.* v. 67.

[3] *Zeit. physiol. Chem.* x. 123.

[4] Schmiedeburg and Schultzen, *Ann. Chem. Pharm.* clxiv. p. 155.

[5] *Ber. d. deutsch. chem. Gesellsch.* xii. 1673.

[6] *Ibid.* viii. p. 811.

CHAPTER IX

THE CARBOHYDRATES

THE carbohydrates form a most important group of organic substances. They are found chiefly in vegetable tissues ; a few are found in the animal organism ; many of the vegetable carbohydrates are used as food for animals, and so they are of importance in a consideration of animal physiology.

The carbohydrates may be conveniently defined as compounds of carbon, hydrogen, and oxygen, the two last named elements being in the proportion in which they occur in water.

They may be for the greater part arranged into three groups, according to their empirical formulæ. The names and formulæ of these groups, and the most important members of each, are as follows :—

1. Glucoses ($C_6H_{12}O_6$)	2. Sucroses or Saccharoses ($C_{12}H_{22}O_{11}$)	3. Amyloses $n(C_6H_{10}O_5)$
+ Dextrose	+ Cane sugar	+ Starch
– Levulose	+ Lactose	+ Glycogen
+ Galactose	+ Maltose	+ Dextrin
Inosite	+ Melitose	Cellulose
– Sorbin	+ Melizitose	Gums
– Eucalin	+ Mycose	Tunicin
—	Synanthrose	—

The + and – sign in the above list indicate that the substances to which they are prefixed are dextro- and lævo-rotatory respectively, as regards polarised light. The formulæ given above are merely empirical ; and there is no doubt that the quantity *n* in the starch group is variable and often large. Investigations relating to the molecular weights of the different carbohydrates have yielded very unsatisfactory results.[1] The most recent work in this direction is that of Brown and Morris.[2] The method these observers adopted was devised by Raoult,[3] and is the outcome of his elaborate investigation into the laws governing the freezing-point of very dilute solutions. Briefly stated, the law is as follows :—When certain quantities of the same substance are successively dissolved in a solvent, on which it has no chemical action, there is a progressive lowering of the freezing-point of the solution, which is proportional to the weight of the substance dissolved in a constant weight of the solvent. It is unnecessary here to describe the actual methods employed, and will be sufficient to quote the principal results obtained by Brown and Morris.

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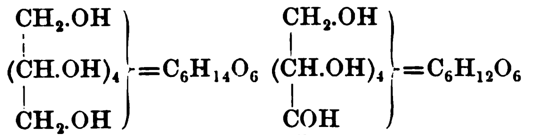
[1] Musculus and Meyer (*Bull Soc. Chim.* (2) xxxv. 370) attempted to determine the relative size of the molecules by observing their rate of diffusion.

[2] *Trans. Chem. Soc.* 1888, p. 610.

[3] *Ann. Chem. Phys.* 1883, 1884, 1885, 1886. *Comptes rend.* 94, 1517; 101, 1056; 102, 1307.

Substance	Formula of Molecule	Molecular Weight
Dextrose[1]	$C_6H_{12}O_6$	180
Cane sugar before inversion . .	$C_{12}H_{22}O_{11}$	342
Cane sugar after inversion . .	$C_6H_{12}O_6$	180
Maltose[2].	$C_{12}H_{22}O_{11}$	342
Arabinose	$C_5H_{10}O_5$	150
Raffinose	$C_{18}H_{32}O_{16}.5H_2O$	594
Galactose	$C_6H_{12}O_6$	180
Inulin	$2(C_{36}H_{62}O_{31})$	1980
Dextrin	$(C_{12}H_{20}O_{10})_6$	1800
Soluble starch	$5(C_{12}H_{20}O_{10})_6$	9000
Starch	It was found impossible to apply the method satisfactorily to starch ; a number of fairly concordant results, however, pointed to a molecular weight of 20,000 to 30,000.	

The carbohydrates are not however simple compounds of carbon with water ; their reactions and derivation products show that their molecular structure is more complicated : they are in fact derivatives of the hexatomic alcohol, mannite. The glucoses may be regarded as the aldehydes of mannite ; they contain in their rational formula the characteristic aldehyde group COH. Thus :—



$$\left.\begin{array}{l} CH_2.OH \\ | \\ (CH.OH)_4 \\ | \\ CH_2.OH \end{array}\right\} = C_6H_{14}O_6 \qquad \left.\begin{array}{l} CH_2.OH \\ | \\ (CH.OH)_4 \\ | \\ COH \end{array}\right\} = C_6H_{12}O_6$$

Mannite Glucose

The sucroses are condensed glucoses, i.e. they are formed by the combination of two molecules of a glucose with the loss of one molecule of water ($C_6H_{12}O_6 + C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$). The amyloses may be regarded as the anhydrides of the glucoses ($C_6H_{12}O_6 - H_2O = C_6H_{10}O_5$).

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[1] This confirms Kiliani's observation that dextrose and levulose yield hydroxy-acids containing seven atoms of carbon.

[2] Cane sugar and maltose are thus isomeric, not polymeric. The difference in their properties must therefore be due to difference of the arrangement of the atoms in their molecules.

By oxidation of the sugars by means of nitric acid, an acid is obtained ; that is, the H_2 removed from the alcohol (mannite) to form the aldehyde (glucose) is replaced by O. The formula of the acid so obtained is therefore $C_6H_{12}O_7$; this is monobasic, and called mannitic acid. On repeating the process, that is, replacing another H_2 by O, we obtain an acid with the formula $C_6H_{10}O_8$; this is dibasic ; of this there are two isomerides, one called *mucic* acid, which is slightly soluble in water, the other *saccharic* acid, which is readily soluble in water. On oxidation some sugars yield one, some the other, acid ; by further oxidation, tartaric acid, then oxalic acid, and finally carbonic acid and water, are obtained. The most important carbohydrates may now be described one by one.

DEXTROSE OR GRAPE SUGAR

This carbohydrate exists in fruits, honey, and in small quantities in the blood, and in numerous tissues, organs, and fluids of the body. It is the form of sugar found in the urine in the disease known as diabetes. It is formed by the hydration of members of the amylose and saccharose group ; such as is brought about by boiling with dilute sulphuric acid.

Dextrose is soluble in hot and cold water, and in alcohol. It is not so sweet as cane sugar. It crystallises from an aqueous solution in white spheroidal masses, and from alcohol in transparent anhydrous prisms. Its solutions rotate the ray of polarised light to the right ; $(\alpha)_D = +56°$ (Hoppe-Seyler).[1]

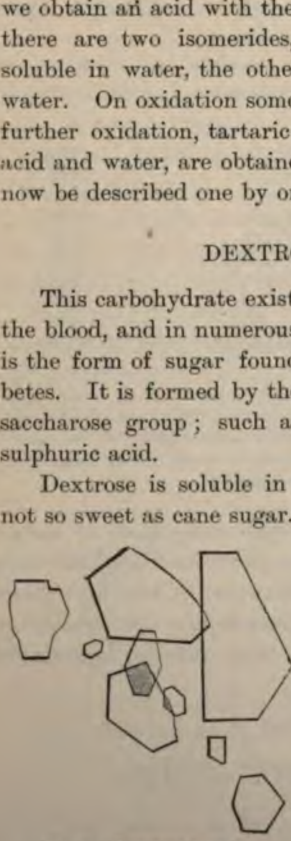

FIG. 41.—Dextrose crystals.

Heated with alkali, dextrose gives a brown or yellow colour due to the formation of glucic and melassic acids.[2]

Nitric acid oxidises dextrose to saccharic acid.

In alkaline solutions, dextrose reduces salts of silver, bismuth, mercury and copper ; in the case of the first three, the metal is precipitated ; cupric are reduced to cuprous compounds, with the separation of cuprous oxide. In the presence of ammonia, dextrose is precipitated by neutral or basic lead acetate.

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[1] Tollens gives $(\alpha)_D = +53{\cdot}1°$. Fresh watery solutions may indicate 104°.

[2] These acids are of doubtful composition. In *Watts' Dictionary* the formula for glucic acid is given as $C_{24}H_{18}O_{18}$, and for melassic acid as $C_{12}H_{10}O_5$. In *Beilstein's Dictionary* the acids formed are stated to be glycic acid ($C_{12}H_{22}O_{12}$) and saccharumic acid ($C_{14}H_{18}O_{11}$).

Dextrose forms compounds with certain acids and bases (e.g. potash, lime): these are called glucosates.

Under the influence of yeast, dextrose is converted into alcohol and carbonic acid ($C_6H_{12}O_6=2C_2H_6O+2CO_2$).[1] It may also undergo the lactic acid fermentation, under the influence of certain bacterial growths.

When sugar is heated to 200°, a brown substance called caramel is formed. This has been separated into three bodies of complex formulæ and doubtful nature (*see* 'Watts' Dict.').

Sugar is also turned brown by the action of sulphuric or hydrochloric acid. This is partly due to charring. A number of other substances, called humous substances by Hoppe-Seyler, have also been separated. These brown products are similar to many produced in vegetable growths naturally, in peat, &c., and are of complex nature and doubtful composition. They have received various names (humin, ulmin, ulmic acid, phlobaphene, tannin-red, hymatomelanic acid, &c.). Among the decomposition products of these substances are formic acid, pyrocatechin, and protocatechuic acid.[2]

The origin, rôle, and destination of dextrose in the body, and other physiological problems connected with its presence, will be more conveniently described with the various tissues and fluids in which it occurs (*see* liver, muscle, blood, urine, diabetes, food, digestion).

Tests for Dextrose.—1. *Trommer's test.*[3]—Add a few drops of dilute cupric sulphate solution to a solution of dextrose and caustic potash or soda in excess. The result is a deep blue solution; the precipitate of cupric hydrate which is formed being soluble in the presence of dextrose. Heat the solution; a little below the boiling-point a red precipitate of cuprous oxide, or a yellow precipitate of cuprous hydrate, is formed. This reduction is due to the formation of glucic and melassic acids which, having a strong affinity for oxygen, take it from the cupric compound.

2. *Fehling's test.*—The principle of this test is the same as that of the preceding. Fehling's solution is thus prepared:—Solution A. Dissolve 36·64 grammes of copper sulphate in 500 c.c. of water. Solution B. Dissolve 173 grammes of sodio-potassium tartrate (Rochelle salts) in 100 c.c. of a solution of caustic soda, having a specific gravity

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[1] Small quantities of glycerine and succinic acid are also formed, and were regarded by Pasteur as derived from the sugar on which the yeast acts (*Ann. Chim. Phys.* (3) xviii. p. 323). v. Udranszky (*Zeit. physiol. Chem.* xiii. p. 539), however, states that the glycerine certainly, and probably the succinic acid, is derived from the substance of the yeast itself, probably from the lecithin it contains.

[2] A full description of humous substances will be found in a paper by Hoppe-Seyler, *Zeit. physiol. Chem.* xiii. 66.

[3] *Ann. Chem. Pharm.* xxxix. (1841) p. 360.

of 1·34, and dilute with water to 500 c.c. These solutions should be kept in well-stoppered bottles, and before using equal volumes of A and B mixed together. The result is a dark blue solution, the Rochelle salt holding the cupric hydrate in solution. The solution should be freshly made, because tartaric acid tends to become converted into its isomeride, racemic acid ; and racemic acid itself reduces cupric salts like sugar. One should always ascertain that it is absent by boiling the Fehling's solution, which should remain unaltered by this treatment. On adding a little solution of sugar and boiling, a red precipitate of the cuprous oxide or hydrate occurs.

3. *Böttger's test.*[1]—Take 5 grammes of basic nitrate of bismuth, 5 grammes of tartaric acid, and 30 c.c. of distilled water. To this add slowly, and with constant stirring, some strong caustic soda solution, until a clear solution is obtained. To a little of this add some solution of dextrose, and boil. A black precipitate of metallic bismuth separates. Or the test may be performed as follows :—The solution of dextrose is mixed with an equal volume of sodium carbonate solution (1 part to 3 of water) ; a few fragments of bismuth subnitrate added, and the mixture boiled. A grey or black precipitate of metallic bismuth is thrown down.

4. *Silver test.*—Add ammonia in excess to a little strong solution of silver nitrate ; add some dextrose and boil, metallic silver is deposited as a mirror at the bottom of the tube. Aldehyde and tartaric acid behave like sugar in this test.

5. *Moore's test.*[2]—Heat the solution of dextrose with a solution of caustic potash. The mixture becomes yellow, and, on exposure to the air, brown from the formation of melassic and glucic acids.

6. *Picric acid test.*—Heat the solution of dextrose with a few drops of solution of picric acid, and heat. Add a little caustic potash, and a brown-red solution is obtained.

7. *Indigo-carmine test.*—A solution of dextrose is rendered faintly blue with indigo-carmine, and faintly alkaline with sodium carbonate. It is then heated to boiling without agitation ; it turns violet, then yellow, but if it is shaken the blue colour is restored.

8. *Fermentation test.*—A test-tube is half filled with solution of dextrose and a little dried German yeast added. Invert the tube over mercury, and leave it in a warm place for 24 hours. The sugar will undergo fermentation; carbonic acid gas accumulates in the tube, and the liquid gives the tests for alcohol. A control experiment should be made with yeast and water in another test-tube, as a small yield of carbonic acid is often obtained from impurities in the yeast.

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[1] Böttger, *Journ. prakt. Chem.* lxx. (1857), p. 432. Nylander, *Zeit. physiol. Chem.* viii. (1884), p. 175.

[2] Moore, *Lancet*, 1844, ii. ; Heller, *Arch. f. mikr. Chem.* vol. i. 1844, p. 292.

9. *The Phenylhydrazine test.*—This test is applied in testing for minute quantities of dextrose, especially in urine. Add a pinch of sodium acetate and a little solution of phenylhydrazine hydrochloride to a solution of dextrose; a yellow precipitate of phenylglucosazone crystals occurring both singly and in bundles forms in a few minutes if the mixture is kept in the water bath at 100° C.; and in a dilute solution of dextrose the crystals should be searched for microscopically (v. Jaksch).[1]

10. *The Saccharimeter test.*—A solution of dextrose rotates the plane of polarised light to the right.

In testing for dextrose, as many tests should be tried as possible, as many other substances give certain of the above tests; for example, reduce copper salts, or rotate the plane of polarised light. The best tests will be found those numbered 2, 3, 8, 9 and 10; and the best of all is 8.

Quantitative determination of Dextrose.—1. *By the Saccharimeter.* This instrument is a polarimeter, and the instruments used and methods adopted are the same as that employed in polarimetric processes generally (*see* pp. 40, 53).

2. *The Fermentation process.* — When mixed with yeast about 95 per cent. of the dextrose in solution is converted into carbonic acid and alcohol. Small quantities of amyl alcohol, glycerine and succinic acid are formed at the same time. The dextrose originally in solution may be estimated either from the loss of weight of the apparatus from the evolution of the gas, or from the gain in weight of an absorption tube containing caustic potash connected with the escape pipe, and which absorbs the carbonic acid. 1 part of $CO_2=2\cdot045$ parts of sugar.

Sir William Roberts devised a simpler process, especially applicable to sugar in urine, in which the sugar present is estimated from the loss of specific gravity a solution undergoes during fermentation. The specific gravity of the solution is accurately taken: yeast is added and after remaining 24 hours in a warm place the specific gravity is again taken. The number of degrees of density lost indicates the number of grains of sugar per ounce; and the percentage is obtained by multiplying the degrees of density by a constant factor. This constant factor is according to Worm-Müller 0·23; according to Manasseïn 0·219. Thus in a urine whose specific gravity before fermentation is 1040, and afterwards 1010, the degrees of density lost = 30, and accordingly 30 grains of sugar are present per ounce, or $30\times0\cdot23=6\cdot9$ per cent.

This method, however, is found practically to give very inaccurate results. The reason of this is, that a constant factor is an impossibility, and, in fact, increases as the percentage of sugar diminishes. For the mathematical demonstration of this fact *see* Budde (Pflüger's *Archiv*, xl. 137).

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[1] *Zeit. klin. Med.* xi. 20, *see* also note on p. 110.

3. *Fehling's method.*—10 c.c. of Fehling's mixture (*see* p. 95) corresponds to 0·05 gramme of sugar.

The solution to be tested should not contain more than about 0·5 per cent. of dextrose. It will be found necessary to dilute strong solutions, and most diabetic urines,[1] with 9 times the amount of water; this must be allowed for in the subsequent calculation.

The solution of dextrose is placed in a burette; and 10 c.c. of Fehling's mixture diluted with 40 c.c. of water, in a white porcelain, dish. The Fehling's mixture is kept constantly boiling, and the sugar is run into it from the burette gradually. The cuprous oxide is thrown down as a red precipitate, and the blue colour of the solution gets less and less, and finally disappears. When the blue colour has gone, the burette is read, and the quantity of solution of sugar used, is that which contains sufficient sugar to reduce 10 c.c. of Fehling's mixture, i.e. 0·05 gramme of sugar.

Suppose 9·5 c.c. of the solution reduced 10 c.c. of Fehling's mixture (=0·05 gramme sugar); then the percentage of sugar $= \frac{0{\cdot}05 \times 100}{9{\cdot}5} = \frac{5}{9{\cdot}5}$ $= 0{\cdot}526$; and if the solution, or the urine had been previously diluted 10 times, the percentage of dextrose in the original solution $= 0{\cdot}526 \times 10$ $= 5{\cdot}26$.

In order to insure accuracy it is always advisable to make a second observation, using the first only as an indication, and proceeding more cautiously. Beginners often find it difficult to determine exactly the point at which the blue colour has completely disappeared. In such a dilemma, a little of the hot fluid should be quickly filtered through a thick filter paper, the filtrate acidulated with acetic acid, and a drop of potassic ferrocyanide added. If copper is present a brown colour or precipitate is produced; in this case, more of the sugar solution must be added, and the operation continued until the filtered hot fluid gives no reaction for copper. Flückiger[2] recommends that a small quantity of calcium chloride should be added before filtering, in order to prevent the mechanical suspension of finely divided cuprous oxide in the solution, and Hagemann[3] has pointed out that in the case of urine, it is necessary to test the first two drops of the filtrate; for by the time the third drop comes through, oxidation of the cuprous oxide has taken place, and cupric oxide is in solution. Such rapid reoxidation does not occur however in solutions of pure dextrose. Hagemann further states that by this method as good results are obtained as by Allihn's method,[1] which is one for determining the amount of copper in the precipitate.

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[1] If the urine is albuminous, the albumin must be first separated by acidulating with dilute acetic acid, boiling, and filtering.

[2] *Zeit. physiol. Chem.* ix. 335.

[3] *Pflüger's Archiv*, xliii. 501.

In making these determinations in urine, it must be borne in mind that other substances may be present which reduce alkaline solutions of copper salts, such as uric acid, creatinine, pyrocatechin, and compounds of glycuronic acid. None of these substances, however, give the fermentation test.

4. *Other Methods.*—Knapp's method[2] is a volumetric one, in which a standard solution of alkaline mercuric cyanide is used. (10 grammes of mercuric cyanide, caustic soda (of sp. gr. 1·14) 100 c.c. made up to a litre with water; 40 c.c. corresponds to 0·1 gramme of sugar). The solution is kept hot, sugar solution run in from a burette, and metallic mercury is deposited. The end of the reaction is the absence of mercury in the fluid; this is ascertained by placing a drop of the clear supernatant fluid on a piece of fine filter-paper, and exposing it to the vapour of ammonium sulphide; when the drop remains unblackened mercury is absent.

Sachsse's method is very similar; the standard solution is mercuric iodide 18 grammes, potassium iodide 35 grammes, caustic potash 80 grammes, water to 1000 c.c.: 40 c.c. corresponds to 0·15 gramme of sugar. The end of the reaction is ascertained by means of drops of a solution of stannous chloride supersaturated with caustic soda, placed on a porcelain dish; as long as the mercuric salt is present, the addition of a drop of the clear supernatant fluid gives with one of these drops a brown colour, or grey precipitate.

Vogel's method is a colorimetric one, and depends on the intensity of the colouration produced by boiling the solution of dextrose with caustic potash. This is compared with a standard solution similarly treated.

Dr. George Johnson has also devised a colorimetric method, depending on the depth of the tint produced by boiling a solution of dextrose with caustic potash and a saturated solution of picric acid, as compared with the tint of a standard.

Pavy's and Gerrard's methods are modifications of Fehling's, and being especially applicable to urine, will be described under that head (*see* Chapter XLV.).

LEVULOSE

When cane sugar is treated with dilute mineral acids, it undergoes a process known as inversion, i.e. it takes up water, and is converted into a mixture of equal parts of dextrose and levulose. Similar hydration changes are produced by ferments, such as the invert ferment of the intestinal juice.

Levulose has been discovered in blood, urine, and muscle. It is uncrystallisable, very soluble in water and in alcohol; it gives the same tests as dextrose, except that it has a powerful lævorotatory action on polarised light. $(\alpha)_D = -106°$.

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[1] *Zeit. anal. Chem.* vol. xxii. p. 248.

[2] *Annal. d. Chem.* vol. cliv. p. 252.

We have seen that dextrose is regarded as an aldehyde; by some, levulose is regarded as the corresponding ketone.

Pure levulose may be obtained by neutralising with lime the mixture of glucoses obtained by the action of sulphuric acid on cane sugar. The levulose lime compound is a solid, while that of dextrose is liquid. By decomposing the lime compound with oxalic acid, pure levulose is obtained.

GALACTOSE

Galactose is formed by the action of dilute mineral acids, or inverting ferments, on lactose or milk sugar. It is dextrorotatory. $(\alpha)_D = +83{\cdot}3^\circ$. Nitric acid oxidises it to mucic acid. Galactose is directly fermentable with yeast; it also reduces Fehling's solution.

INOSITE

Inosite is a glucose which is found in muscle, kidney, liver, nervous tissues, and several other organs of the body. It has also been separated in small quantities from the blood, traces exist in most diabetic urines, in the urine of certain cases of Bright's disease, and according to Choetta,[1] Gallois,[2] and Külz,[3] in normal urine too.

It is also obtained from peas, beans, lentils, potato, asparagus, dandelion, foxglove, and many other plants.

Preparation.—From beans. A watery extract is evaporated to a syrup, and precipitated with alcohol; the precipitate is dissolved in water, and the inosite allowed to crystallise out.

From muscle or other tissues.[4] An aqueous extract is freed from albumin by acidulation, boiling and filtering; from phosphates by the addition of baryta water and filtering. The filtrate is concentrated, and creatine crystallises out. The mother liquor is boiled with four times its volume of alcohol, and the precipitate so formed is removed. The clear liquid is set aside for twenty-four hours, and crystals of inosite often separate; if not, ether is added, and the mixture shaken, inosite then separates in lustrous leaflets. It is purified by recrystallisation.

From urine. Take several litres of urine, and add neutral then basic lead acetate. Collect the precipitate produced by the latter; decompose it with sulphuretted hydrogen; filter; evaporate the filtrate to a syrup, and add alcohol and ether. Inosite crystallises out.

Properties.—It forms large colourless monoclinic prisms, often grouped in rosettes. The crystals contain two molecules of water of crystallisation. It has a sweet taste, is soluble in water, but not in absolute alcohol, or ether.

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[1] *Ann. Chem. Pharm.* xcix. p. 289.

[2] *De l'inosurie, Thesis*, Paris, 1864.

[3] *Centralbl. f. Med. Wiss.* 1875, p. 933.

[4] Boedeker, *Ann. Chem. Pharm.* vol. cxvii. p. 118.

It is precipitated by a mixture of basic lead acetate and ammonia. It is capable of the lactic acid fermentation[1] but not of the alcoholic. Its solutions have no action on polarised light; it does not reduce metallic oxides; it gives no change of colour when boiled with caustic potash, neither is it decomposed by weak acids.

Tests.—(1) Evaporate a little of its solution with a little nitric acid on a platinum dish; treat the residue with a little ammonia and calcium chloride, and evaporate to dryness at a gentle heat. A bright red or violet colour is produced. This test only succeeds with pure solutions (Scherer).[2]

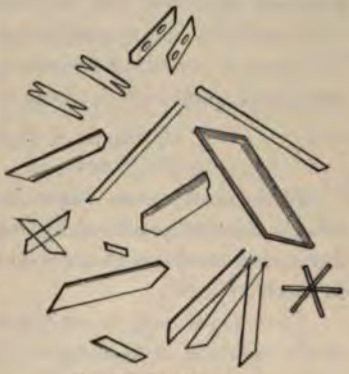
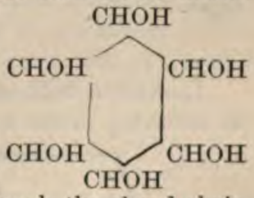


FIG. 42.—Inosite crystals.

(2) Add a little mercuric nitrate to a solution of inosite, on a porcelain dish; a yellow precipitate is produced. On heating this gently, it will become red; on cooling the colour vanishes. Proteids, tyrosine, and sugar must be absent (Gallois).

Constitution.—From a study of its nitro-substitution and other products, Maquenne[3] concludes that the graphic formula for inosite may be thus represented. It is in other words a hexatomic alcohol with six secondary alcohol groups arranged in a ring; this symmetrical construction excluding any power to rotate polarised light according to the theory of Le Bel and Van 't Hoff (*see* p. 45). It is not an aldehyde, nor an acetone, nor a polyphenol, though the closed chain suggests an aromatic structure.



CANE SUGAR

This sugar is generally distributed throughout the vegetable kingdom in the juices of plants and fruits, especially the sugar cane, beetroot, mallow, and sugar maple. It is a substance of great importance as a food; after abundant ingestion of cane sugar, traces may be found in the blood and urine, but the greater part undergoes inversion.

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[1] According to Hilger (*Ann. Chem. Pharm.* vol. clx. p. 333) the variety of acid formed is sarcolactic.

[2] *Ann. Chem. Pharm.* vol. lxxiii. p. 322.

[3] *Compt. rend.* civ. (1887), 225, 297, 1719, 1853.

Pure cane sugar holds cupric hydrate in solution in an alkaline liquid, i.e. with Trommer's test it gives a blue solution. But no reduction occurs on boiling.[1] It crystallises in monoclinic prisms. Aqueous solutions are dextrorotatory. $(\alpha)_D = +73 \cdot 8°$. By boiling with water, or more readily by boiling with dilute mineral acids, or by means of inverting ferments, it undergoes inversion, i.e. it takes up water and splits into dextrose and levulose.

$$\underset{\text{[cane sugar]}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{[dextrose]}}{C_6H_{12}O_6} + \underset{\text{[levulose]}}{C_6H_{12}O_6}$$

With yeast, cane sugar is first inverted by means of a special soluble ferment produced by the yeast cell, and then there is an alcoholic fermentation of the glucoses so formed. Nitric acid oxidises cane sugar to saccharic acid.

Cane sugar may be estimated in the following way:—Take 40 c.c. of the solution of cane sugar; add 1 c.c. of a 25 per cent. solution of sulphuric acid, and boil for half-an-hour. Care must be taken not to char the sugar. Bring the solution of sugar to its original volume by adding water. Place it in the burette, and run it into boiling Fehling's solution, as in the estimation of dextrose. It may be necessary to add excess of soda or potash to the Fehling's solution, so that the sulphuric acid in the sugar solution may be fully neutralised. Every 95 parts of glucose found corresponds to 100 parts of cane sugar.

LACTOSE

Lactose or milk sugar occurs in milk. It has also been described as occurring in the urine of women in the early days of lactation or after weaning.

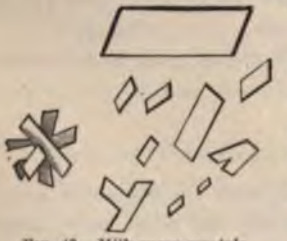

FIG. 43.—Milk sugar crystals.

It crystallises in rhombic prisms, which contain a molecule of water of crystallisation. It is soluble in six parts of cold, and 2½ parts of hot, water. It is thus much less soluble than cane sugar or dextrose. It has only a faint sweet taste. Aqueous solutions are dextrorotatory. $(\alpha)_D = +59 \cdot 3°$. It is insoluble in alcohol and in ether.

Solutions of lactose reduce Fehling's solution, but less powerfully than dextrose. If it required seven parts of a solution of dextrose to reduce a given quantity of Fehling's solution, it would require ten parts of a solution of lactose of the same strength to reduce the same quantity of Fehling's solution.

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[1] Most specimens of commercial cane sugar contain other forms of sugar as impurities, and these cause a small amount of reduction.

By boiling with water, or more readily by boiling with dilute acids, or by means of inverting ferments, as in the alimentary canal, it takes up water and is converted into a glucose called galactose:

$$\underset{\text{[lactose]}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{[galactose]}}{2C_6H_{12}O_6}$$

With yeast, lactose is first inverted to galactose, and with this the alcoholic fermentation takes place ; but this occurs slowly. With the lactic acid organism, that which brings about the souring of milk, the lactic acid fermentation is produced ; this may also occur as the result of the action of putrefactive bacteria, e.g. in the alimentary canal. The lactic acid fermentation consists of the two stages represented by the following equations :—

$$(1).\quad \underset{\text{[lactose]}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{[lactic acid]}}{4C_3H_6O_3}$$

$$(2).\quad \underset{\text{[lactic acid]}}{2C_3H_6O_3} = \underset{\text{[butyric acid]}}{C_{11}H_8O_2} + 2CO_2 + 2H_2$$

Nitric acid oxidises lactose to mucic acid.

To detect lactose in milk.—Acidulate slightly with acetic acid, boil, filter, and test the filtrate with Fehling's solution, or by Böttger's bismuth test.

To prepare lactose from milk.—Acidulate with acetic acid to precipitate the casein and fat ; filter ; boil again to precipitate albumin and filter again ; evaporate the filtrate to a small bulk ; set aside to crystallise ; the crystals may be purified by recrystallisation.

MALTOSE

Maltose is the end product of the action of malt-diastase on starch, and can also be formed as an intermediate product in the action of dilute sulphuric acid on the same substance. It also appears to be the chief sugar formed from starch by the diastatic ferments contained in the saliva (ptyalin) and pancreatic juice (amylopsin).

Maltose can be obtained in the form of acicular crystals ; aqueous solutions are strongly dextrorotatory. $(\alpha)_D = +150°$. Solutions of maltose reduce alkaline solutions of copper, bismuth and other metallic salts ; but its reducing power as measured by Fehling's solution is one third less than that of dextrose.

By prolonged boiling with water, or more readily by boiling with a dilute mineral acid, or by means of an inverting ferment, such as occurs in the intestinal juice, it is converted into dextrose.

$$\underset{[\text{maltose}]}{C_{12}H_{22}O_{11}} + H_2O = \underset{[\text{dextrose}]}{2C_6H_{12}O_6}$$

It undergoes readily the alcoholic fermentation.

STARCH

Starch is widely diffused through all parts of the vegetable world. It occurs in nature in the form of microscopic granules ; these vary in appearance and size according to their source, but each consists of a central spot around which are more or less parallel rings of starch proper or granulose, alternating with layers of cellulose. A variety of granulose is present in small amount, which gives a red colour with iodine ; it is called erythro-granulose (Brücke).

It is nearly insoluble in cold water. When boiled with water, the granules burst, and an imperfect opalescent solution is formed. If concentrated, this gelatinises on cooling. It is also insoluble in alcohol and in ether. It is a colloid substance ; that is, it does not pass through animal membranes, or vegetable parchment.

Tests.—(1) With iodine it gives a blue colour, which disappears on heating, the iodide of starch being dissociated at a high temperature : on cooling it reappears. In performing this test, care must be taken not to apply heat for too long a time, or all the iodine is driven off and consequently no blue colour reappears on cooling.

(2) Tannic acid gives a yellow precipitate, which dissolves on heating.

(3) Solutions of starch are dextrorotatory. $(\alpha)_D = +216°$.

Decompositions.—At 200° C. dry starch, at 160° C. solutions of starch, are changed into dextrin. Prolonged heating changes it into dextrose. It is rapidly converted into dextrose by heating it with dilute mineral acids. It may in this way be estimated quantitatively, 90 parts of the dextrose so formed corresponding to 100 parts of starch. By the action of diastatic ferments,[1] maltose is, as we have seen, the chief end product ; here also dextrin is an intermediate stage. When starch is converted into dextrose by the action of acids, the following equation represents what occurs :—

$$\underset{[\text{starch}]}{3C_6H_{10}O_5} + H_2O = \underset{[\text{dextrose}]}{C_6H_{12}O_6} + \underset{[\text{achroo-dextrin}]}{C_6H_{10}O_5} + \underset{[\text{erythro-dextrin}]}{C_6H_{10}O_5}$$

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[1] Raw starch is less readily acted on by ferments than boiled starch, and the starches from different plants vary much in digestibility.

If some starch solution be boiled with dilute mineral acids, or be acted on by diastatic ferments, the solution will become clear from the formation of a polymeride of starch, called soluble starch or amidulin; this like the original solution gives a blue colour with iodine; as the action progresses the blue colour becomes less and less intense, then mixed with a red tint to form violet, and then replaced by the red tint due to the presence of a form of dextrin called erythrodextrin. If the liquid be tested with Fehling's solution, reduction will show the presence of dextrose or maltose. Dextrin and sugar are produced simultaneously (*see* above equation). Testing the liquid a little later, the reduction with Fehling will be found much more abundant, and testing with iodine there will be no colouration: this is due to the conversion of erythro-dextrin into sugar. If alcohol be added, a precipitate is produced; this consists of a form of dextrin which gives no colour with iodine, and which is consequently called achroo-dextrin. It is converted ultimately into sugar also, but with greater difficulty than is erythro-dextrin.

The formation of maltose from starch may be represented by the following equation (Brown and Morris):—

$$\underset{\text{[starch]}}{10(C_6H_{10}O_5)_n} + 4_nH_2O = \underset{\text{[maltose]}}{4_nC_{12}H_{22}O_{11}} + \underset{\text{[dextrin]}}{(C_{12}H_{20}O_{10})_n}$$

DEXTRIN

Dextrin is the name given to the intermediate products in the hydration of starch, and Brücke distinguishes two varieties: erythro-dextrin, which gives a red-brown colour with iodine, and achroo-dextrin, which does not. It is said to occur in small quantities in blood, muscle, and liver; also occasionally in diabetic urine (Reichardt).[1]

Dextrin is readily soluble in water, but insoluble in alcohol and ether. It is gummy and amorphous. It does not reduce Fehling's solution, nor does it ferment with yeast. It has a strong dextrorotatory action. $(\alpha)_D = +138{\cdot}88°$. By hydrating agencies it is converted into glucose.

Dextrin gives the same, or a very similar, colour with iodine as glycogen does. It may be distinguished from glycogen by the want of opalescence of its solutions, and by its not being precipitable by basic acetate of lead. It is, however, precipitable by basic acetate of lead and ammonia.

Dextrin gives also a very similar colour to that given by erythro-granulose. That dextrin and erythro-granulose are not identical is shown by their having a different affinity for iodine. If a small quantity of iodine be added to a mixture of dextrin and starch, a red-brown solution is first formed; this on the addition of more iodine becomes violet from the formation of the blue iodide of starch. If on the other hand a small quantity of iodine be added to a mixture of erythro-granulose and starch, a blue solution is formed first; this on the addition of more iodine becomes violet from the formation of the red iodide of erythro-granulose. All these iodides undergo dissociation at a high temperature, that is, the colour disappears on heating, and reappears as the liquid cools.

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[1] Reichardt, *Zeit. Anal. Chem.* 1875.

In the recent work of Brown and Morris on the molecular weights of the carbohydrates, results have been obtained which show that the formation of dextrin is a more complex process than has been hitherto considered. They assign to a substance formed in one stage in the reaction (malto-dextrin) the formula $C_{12}H_{22}O_{11}(C_{12}H_{20}O_{10})_6$, which may be regarded as constituted of one *amylon* or maltose group, in combination with six *amylin* or dextrin groups. Its molecular weight would on this supposition be 2286.

Precipitation of Colloid Carbohydrates by Salts.—The use of neutral salts in the precipitation and separation of proteids is also applicable to colloid carbohydrates. Pohl[1] has examined a number of these, and finds that some, like gum arabic, are not precipitable by saturating their solutions with neutral salts; others, like gum tragacanth, are precipitated by saturation with ammonium sulphate; and others again, like soluble starch and dextrin, are precipitated by saturation with sodium sulphate, magnesium sulphate, and ammonium sulphate.

I have myself found that glycogen is precipitated by saturation with ammonium sulphate and magnesium sulphate, but not at all or only very slightly by sodium chloride.

GLYCOGEN

Glycogen or animal starch is found in the liver, muscle, placenta, white blood corpuscles, cartilage cells, and in embryonic tissues generally. It has also been found in some specimens of diabetic urine (Leube).[2] It is a substance of great physiological importance. The methods of preparing it and estimating its quantity, together with a discussion of its functions, will be given under liver and muscle, the two tissues in which it occurs in the adult in the greatest quantity.

Glycogen is a white, tasteless, odourless powder, soluble in water, forming a densely opalescent solution. It is insoluble in alcohol and in ether. It is strongly dextrorotatory; $(\alpha)_D = +211°$. With Trommer's test it gives a blue solution, but no reduction occurs on boiling.

Glycogen gives with iodine a port-wine red colour; this easily distinguishes it from starch. The colour disappears on heating, and reappears as the liquid cools. The colour which dextrin gives with iodine is very similar, but dextrin forms a clear, not an opalescent, solution with water, and is not precipitated by basic lead acetate, as is glycogen.

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[1] *Zeit. physiol. Chem.* xiv. 151.

[2] *Virchow's Archiv*, vol. cxiii. 391.

Prolonged boiling with water or boiling with dilute mineral acids converts glycogen into dextrose. The ferments of the salivary glands, of the pancreas, and glycerine extracts of liver and other organs change glycogen into maltose, intermediate products of the nature of dextrin being formed in each case, as with starch. During the processes of metabolism that occur normally in the liver, glycogen is not only stored up in the cells, but a certain amount of the stored glycogen is transformed into sugar and leaves the liver by the hepatic vein. The form of sugar so formed is not maltose but dextrose.

CELLULOSE

This is the colourless material which composes the cell-walls and woody fibre of plants[1]; it may be obtained in the pure state from cotton or linen fibre by boiling out impurities with alkali, alcohol, and ether.

It is insoluble in water, alcohol, or ether, but dissolves in an ammoniacal solution of cupric oxide. From this solvent it may be recovered in an unchanged form.

By the action of strong sulphuric acid, cellulose is converted either into an insoluble substance which colours blue with iodine, or into a soluble substance of the nature of dextrin. A useful material called vegetable parchment is prepared by dipping sheets of paper into strong sulphuric acid. By boiling with dilute sulphuric acid cellulose is converted into dextrose. The various digestive ferments have little or no action on cellulose.

Cellulose is, however, not confined to vegetable tissues. It is the chief constituent of the test or outer investment of the Tunicates, and is sometimes called tunicin. Schäfer[2] found that the cellulose obtainable from the mantles of the Pyrosomidæ, Salpidæ, and Phallusia mammilaris has the same elementary composition as vegetable cellulose, and has also identical properties; for instance, it dissolves in cuprammonia, and is converted by nitric acid into an explosive nitrate soluble in ether (gun-cotton).

According to Berthelot[3] tunicin differs from cellulose in being less easily convertible into dextrose by the action of dilute sulphuric acid.

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[1] The different varieties of cellulose will be found described in *Watts' Dict. of Chem.* vol. i. 1888.

[2] *Annal. Chem. Pharm.* clx. 312.

[3] *Ann. de Chem. et de Phys.* Sér. 3, tome 56, p. 153.

The skin of the silk-worm is stated to contain cellulose (De Lucca).[1]

The mucilaginous investing matrix or zoocytium which surrounds the colonies or social clusters formed by the protozoon *Ophrydium versatile* is also composed of cellulose (Halliburton).[2]

Virchow[3] found cellulose in degenerated human spleen, and in certain parts of the brain, and more recently Freund[4] has found it in the blood and tissues in cases of phthisis.

GUMS

Gum arabic, the natural exudation from several species of acacia, consists chiefly of the potassium and calcium compounds of arabin, or arabic acid $(C_6H_{10}O_5)_n$.

Arabin forms a thick sticky solution with water; it is insoluble in alcohol and in ether. With copper sulphate a thick gelatinous precipitate is formed, which is a compound of cupric oxide and the gum. This is insoluble in caustic soda, and is not reduced on boiling. With ferric chloride a similar thick precipitate is formed.

When boiled with dilute sulphuric acid, arabin yields a crystalline sugar called arabinose; this has the exceptional formula $C_5H_{10}O_5$. It is strongly dextrorotatory, reduces alkaline solutions of cupric hydrate (Fehling's solution), but will not undergo the alcoholic fermentation. Arabin is oxidised by nitric acid to mucic acid.

Dextrin or British gum.—See p. 105.

Animal gum.—This substance was discovered by Landwehr, and is a constituent of mucin. It has long been known that when mucin is boiled with dilute sulphuric acid it yields a reducing but unfermentable sugar $(C_6H_{12}O_6)$. This sugar comes from the animal gum which is present in mucin in combination with a proteid. Animal gum is sticky, gives gelatinous precipitates with copper and iron salts, and has the same empirical formula, $(C_6H_{10}O_5)_2$, as vegetable gum. Fuller particulars regarding its properties and physiological importance will be found under mucin, connective tissue, cartilage, &c.

Animal dextran, $(C_6H_{10}O_5)_n$, a gum-like substance secreted by the *Schizoneura lanuginosa*, a gall-producing louse that attacks elms. $(\alpha)_D = +156{\cdot}7^\circ$ (Liebermann).[5]

OTHER CARBOHYDRATES

Melitose, from eucalyptus manna. *Melizitose*, from larch manna. *Mycose* or *Trehalose*, from ergot.

These are all sucroses; they are dextrorotatory, and do not reduce alkaline cupric solutions.

Synanthrose is also a sucrose; it is found in the roots of certain plants. It has no action on polarised light.

Sorbin, from sorbic acid. *Scyllite*, from the intestines of the hag-fish and skate. *Eucalin*, arising from the fermentation of melitose.

These three glucoses are nearly allied in their properties to inosite.

Inulin is a lævorotatory carbohydrate found in the roots of certain plants together with synanthrose. In the pure state it forms characteristic crystalline spherules. When boiled with dilute acids it yields levulose. Its formula is $x(C_{36}H_{62}O_{31})$.

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[1] *Compt. rend.* lii. p. 102; lvii. p. 43.

[2] *Quart. J. Mic. Science*, July, 1885.

[3] *Compt. rend.* xxxvii. 492, 860.

[4] *Wiener med. Jahrb.* 1886, 335.

[5] *Pflüger's Archiv*, xl. 454.

Raffinose, a crystalline carbohydrate which can be separated from molasses. Its formula is $C_{18}H_{32}O_{16}$; the crystals contain five molecules of water of crystallisation.

Dextrane, $C_6H_{10}O_5$, a gummy substance occurring in unripe beetroot (Scheibler).[1]

Lichenin, $(C_6H_{10}O_5)_n$, occurs in the intercellular substance of Iceland moss and certain algæ.

Paramylum (Zooamylum : Bütschli), $(C_6H_{10}O_5)_n$, occurs in the form of granules resembling starch in the infusorian, *Euglena viridis*, and in all gregarinæ.[2]

Paragalactin and other insoluble carbohydrates in the cell membrane of seeds which occur there with cellulose, differ from it in being insoluble in ammoniacal solutions of copper oxide (Schulze).[3]

GLUCOSIDES

The substances constituting this class occur in many plants, and a few are found in animals also. They yield on decomposition a carbohydrate, generally a glucose, together with other substances.

Amygdalin in bitter almonds may be taken as an example. In the bruised almond a ferment called emulsin or synaptase produces from it bitter almond oil, hydrocyanic acid, and glucose :—

$$\underset{\text{[amygdalin]}}{C_{20}H_{27}NO_{11}} + 2H_2O = \underset{\text{[hydride of benzoyl]}}{C_7H_6O} + \underset{\text{[hydrocyanic acid]}}{HCN} + \underset{\text{[glucose]}}{2C_6H_{12}O_6}$$

The following equations represent the decompositions of a few other important vegetable glucosides :—

$$\underset{\text{[salicin]}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{[saligenin]}}{C_7H_8O_2} + \underset{\text{[glucose]}}{C_6H_{12}O_6}$$

$$\underset{\text{[tannin]}}{C_{27}H_{22}O_{17}} + 4H_2O = \underset{\text{[gallic acid]}}{3C_7H_6O_5} + \underset{\text{[glucose]}}{C_6H_{12}O_6}$$

$$\underset{\text{[potassium myronate]}}{C_{10}H_{19}KNS_2O_{10}} = \underset{\text{[hydrogen potassium sulphate]}}{\left.\begin{matrix}H\\K\end{matrix}\right\}SO_4} + \underset{\text{[oil of mustard]}}{\left.\begin{matrix}CS\\C_3H_5\end{matrix}\right\}N} + \underset{\text{[glucose]}}{C_6H_{12}O_6}$$

Among other important vegetable glucosides are digitalin, ruberythric acid (which yields alizarin), coniferin (which yields vanilin), and indican[4] (which yields indigo, *see* p. 78).

The animal glucosides are: (1) mucin, which yields a proteid and animal gum; (2) cerebrin (*see* nervous tissues); (3) chitin (*see* epithelial structures); (4) carminic acid (*see* pigments).

GLYCURONIC ACID

Glycuronic acid is a substance which occurs under certain circumstances in urine, and from the fact that it rotates polarised light to the right ($(\alpha)_D = +19$), and reduces alkaline solutions of copper oxide, is apt to be mistaken for dextrose.

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[1] *Jahresb. f. Chem. Technologie*, 1875, 790.

[2] Maupas, *Compt. rend.* cii. 120.

[3] *Ber. deutsch. chem. Gesell.* xxii. 1192.

[4] The indican of urine is, however, not a glucoside, *see* p. 79.

Its formula is $C_6H_{10}O_7$, and it is no doubt related to the carbohydrate When pure it is not crystalline, but its anhydride, $C_6H_8O_6$, forms colourless acicula crystals. It reduces not only Fehling's solution, but also gives Böttger's bismuth test. It does not, however, undergo the alcoholic fermentation, and can by this means be easily distinguished from sugar. It is insoluble in ether, but readily soluble in water and hot alcohol, crystallising out from the latter on cooling. It is precipitable from an aqueous solution by baryta water as an insoluble baryta compound. Though related in its composition so nearly to the carbohydrates, it yields with urea, decomposition products which are aromatic, such as orthonitrobenzyl alcohol (Jaffe).[1] It occurs in the urine in the form of the potassium salt ($C_6H_9O_7K$). It is found there after the administration of certain drugs (chloral and butylchloral,[2] nitrobenzol,[3] ortho-nitro toluol,[4] camphor,[5] &c.). It also occurs in the urine after chloroform narcosis, and in the paralytic secretion that takes place on section of the renal nerves.[6] Potassium glycuronate, like dextrose, gives with phenylhydrazine hydrochloride a crystalline precipitate.

Bromine converts glycuronic acid into saccharic acid, thus showing the presence in the former acid of an aldehyde group, and also its close relation to dextrose (Thierfelder).[7] Schmiedeberg and Meyer[8] consider that it arises from the dextrose in the body; Külz[9] has suggested that it may originate from inosite. (*See* also Urine.)

NOTE

Hirschl ('Zeit. physiol. Chem.' xiv. 377) states in reference to the phenylhydrazine test for sugar, more especially in urine, that it is as trustworthy as the fermentation and polarimeter tests. The mixture should, however, remain in the water-bath at least an hour before crystals are looked for. The fine bright yellow needles of phenylglucosazone (melting-point 204° C.), either single or in stars, are then easily distinguishable from the brownish amorphous precipitate (melting-point 150°) which glycuronic acid gives. If the mixture be left in the water-bath for a shorter time than an hour, the glycuronic acid compound formed is crystalline acid, and is liable to be mistaken for phenylglucosazone.

In addition to dextrose, three other sugars have been described in human urine:—(1) Levulose (Zimmer, 'Deutsch. med. Woch.' ii. 329; Seegen, 'Centralbl. med. Wiss.' xxii. 753); this has been found only in diabetics mixed with dextrose, from which it is distinguishable only by the polarimeter; (2) Lactose (Hofmeister, 'Zeit. physiol. Chem.' i. 101); this is found in the urine of suckling women; the crystals of phenyllactosazone, formed by the action of phenylhydrazine, are ten times wider than those of phenylglucosazone, and melt at 200°; (3) Maltose, found in diabetic urine by Le Nobel, gives a precipitate of phenylmaltosazone which occurs in yellow tables melting at 82° C.

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[1] *Zeit. physiol. Chem.* ii. 47.

[2] Musculus and v. Mering, *Pflüger's Achiv*, xx. 64.

[3] v. Mering, *Centralbl. Med. Wiss.* 1875, No. 55.

[4] Jaffe, *loc. cit.*

[5] Schmiedeberg and Meyer, *Zeit. physiol. Chem.* iii. 422.

[6] Ashdown, *Brit. Med. Journ.* vol. i. 1890, p. 171.

[7] *Ber. deutsch. Chem. Gesellsch.* xix. p. 3148.

[8] *Zeit. physiol. Chem.* iii. p. 437.

[9] *Zeit. Biol.* xxiii. p. 475. In this paper will be found a method of preparing glycuronic acid from purree (a yellow substance probably obtained from the urine of camels who have eaten certain fruits); *see* also Thierfelder, *Zeit. physiol. Chem.* xi. 388.

CHAPTER X

THE PROTEIDS

The proteids are the most important substances that occur in animal and vegetable organisms; none of the phenomena characteristic of life occur without their presence. They are invariable and constant constituents of protoplasm.

The term proteids was originally given to these substances by Mulder (πρωτεῖον, pre-eminence), and the name is a convenient one in which to include all the heterogeneous members of the group. It must not be supposed that in adopting Mulder's nomenclature we in any way accept Mulder's theory of the constitution of proteids, which will be referred to later.

The expressions 'proteid' and 'albuminous substance' are synonymous. The word 'albumin' is restricted now to a definite class of proteids. The word 'albuminoid' should be restricted to a class of compounds (gelatin, mucin, &c.), which, although having certain resemblances to the proteids, differ from them in many important points. The words albuminate, albumose, albumid, &c., are applied to certain derivatives of the proteids; these terms should always be most carefully used, as their similarity to one another is apt to give rise to confusion.

The following short description of the proteids must serve in lieu of a logical definition; for although the proteids are the most important of all organic substances, they are those about which we have the least information.

'Proteids are highly complex and (for the most part) uncrystallisable compounds of carbon, hydrogen, oxygen, nitrogen, and sulphur, occurring in a solid viscous condition, or in solution in nearly all the solids and liquids of the organism. The different members of the group present differences in physical and to a certain extent even in chemical properties. They all possess, however, certain common chemical reactions, and are united by a close genetic relationship (Gamgee).[1]

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[1] *Physiol. Chem.* p. 4.

The following table from Gorup-Besanez[1] exhibits the proportion of proteids contained in the liquids and solids of the body :—

	Per cent.		Per cent.
Cerebro-spinal liquid	0·09	Chyle	4·09
Aqueous humour	0·14	Blood	19·56
Liquor amnii	0·70	Spinal cord	7·49
Intestinal juice	0·95	Brain	8·63
Liquor pericardii	2·36	Liver	11·64
Lymph	2·46	Thymus	12·29
Pancreatic juice	3·33	Muscles	16·18
Synovia	3·91	Tunica media of arteries	27·33
Milk	3·94	Crystalline lens	38·30

The proteid constituents of the animal body are derived from vegetables, either directly, or indirectly through the body of another animal. Synthetic processes do occur in the animal body, but to a much greater extent in vegetables. Here the proteids are built up from simpler compounds derived ultimately from the soil and the atmosphere. In animals the proteids are first converted into substances called peptones, in which form they are absorbed ; the peptones are reconverted into proteids similar to those originally ingested, and these proteids are assimilated, that is, become part of the living organism. During life, however, there is not only a process of building up going on, but also a process of breaking down, the two constituting what is known as metabolism. The result of the destructive metabolism of proteids is the formation of various oxides, carbonic acid and water, and certain not fully oxidised products (urea, uric acid, &c.) which contain the nitrogen of the original proteid.

COMPOSITION AND CONSTITUTION OF THE PROTEIDS

The various proteids differ a good deal in elementary composition. Hoppe-Seyler gives the following percentages :—

	C	H	N	S	O
From	51·5	6·9	15·2	0·3	20·9
To	54·5	7·3	17·0	2·0	23·5

From figures of this kind various observers have attempted to construct an empirical formula for certain typical proteids, egg-albumin being the one usually selected. Thus Lieberkühn assigned to albumin the formula $C_{72}H_{112}N_{18}O_{22}S$; Loew[2] gives the same formula ; Harnack[3] gives $C_{204}H_{322}N_{52}O_{66}S_2$; Schützenberger[4] $C_{240}H_{392}N_{65}O_{75}S_3$, and there have been others. The great divergence between these numbers requires no comment.

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[1] *Lehrbuch*, p. 128.

[2] Loew and Bokorny, *Die chemische Kraftquelle im lebenden Protoplasma*, Munich, 1882.

[3] *Zeit. physiol. Chem.* v. 207.

[4] *Bull. Soc. Chim.* vols. xxiii. and xxiv.

Results which are equally conflicting have been obtained in attempts to ascertain the molecular weight of albumin. Lieberkühn, in 1852, attempted to establish it by analysing the copper compound resulting from the action of a soluble copper salt on a solution of egg-albumin. This compound has since then been analysed by six different investigators and found to contain from 1·5 to 5·2 per cent. of CuO. The compound formed is thus one which contains no definite quantity of copper, or there may be several copper albuminates in the mixture. Chittenden and Whitehouse[1] have both with egg-albumin and myosin found equally variable results with other metals. Therefore, although the molecular weight of albumin is undoubtedly very high, no accurate measurements have as yet been made.

Still more contradictory and mutually destructive theories have been formed with regard to rational formulæ for the proteids. The usual method which a chemist follows in attempting to discover the constitution of any substance is first to observe the way in which it decomposes under certain circumstances (analysis), and then if possible to build up the original material from the simpler compounds so obtained (synthesis). In the case of the proteids there have been many observations of the nature of analysis, but synthesis has not yet been successful. The various theories that have been formed all depend on the results of the decomposition of proteids, and here we meet with many difficulties. First, because the products of decomposition are so numerous; secondly, because under differing circumstances they are so various; and, thirdly, because in all probability living proteid differs in its constitution from the non-living proteid, with which necessarily laboratory experiments have to be made. Metabolism is a very different process in its results from those of experimental chemistry. Before going into the theories themselves it will be necessary to give a list of the products of decomposition which result from different treatment of albumin.

(1) In the body. Carbonic acid, water, urea are the chief final products. Glycocine, leucine, uric acid, &c., are probably intermediate products. Carbohydrates (glycogen) and fats may also originate from proteids.

(2) Action of heat. The oily liquid (Dippel's oil) obtained by dry distillation contains ammoniacal salts of the fatty acids, amines, and aromatic compounds.

(3) Putrefaction. Ammonia, ammonium sulphide, carbonic acid,

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[1] *Studies from the Lab. Physiol. Chem. Yale Univ.* ii. 95.

volatile fatty acids, lactic acid, and amido-acids (leucine, tyrosine, &c.). Indole and skatole.

(4) Action of strong mineral acids and caustic alkalis. The chief products are leucine, tyrosine, aspartic acid, and glutamic acid.

(5) Action of baryta water in sealed tubes. (*See* further Schützenberger's theory, next page.)

(6) Action of oxidising agents. With nitric acid a yellow substance called xanthoproteic acid is first formed. As the constitution of albumin itself is unknown, that of its compounds is much more involved in obscurity. In spite of this, various substances have been prepared as the result of the action of nitric acid on albumin, and names trinitro-albumin, hydroxytrinitro-albumin, hexnitro- and hexamido-albumin sulphonic acids,[1] &c., with formulæ have been given to them. It need hardly be said how exceedingly uncertain all this is, and that different analysts give different results. By oxidation with potassium permanganate, Maly[2] obtained a substance to which he gave the name oxyprotosulphonic acid. These substances on further oxidation break up into simpler compounds like those already enumerated (fatty acids, amido-acids, aromatic bodies).

From results such as these, in which we see that amides, aromatic substances, and fatty derivatives are the most abundant, Gautier[3] concludes that the different proteids differ in the arrangement, relation, proportion, and in some cases even in the nature of their contained radicles.

We can now pass on to consider briefly the various theories that have been held with regard to the constitution of the proteid molecule.

a. Mulder's theory.—Mulder[4] observed that by the action of caustic potash, sulphur was removed from a proteid, and he called the sulphur-free residue *protein*, and ascribed to it the formula $C_{36}H_{26}N_4O_{10}$. He considered that the different proteids were combinations of 'protein' with different amounts of sulphur. Liebig and others pointed out that the warming of a proteid with potash removes not only sulphur, but also ammonia ; and even though the residue gives no further colour with lead salts, it still retains some sulphur. It is thus possible to speak of two forms of sulphur in proteid, that which is loosely and that which is firmly combined.[5] Further investigation has clearly shown that

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[1] Loew, *J. pr. Chem.* (2) iii. 180.

[2] *Centralbl. med. Wiss.* 1885, 740. *Maly's Jahresb.* xviii. 10.

[3] *Chimie appliquée à la physiol.* i. 253.

[4] *Ann. Chem. Pharm.* lxi. 121.

[5] Danilewsky, *Zeit. physiol. Chem.* vii. 440. A. Krüger, *Pflüger's Archiv*, xliii. 244. In the latter paper will be found an interesting series of suggestions as to the way in which these two forms of sulphur are combined.

'protein' is an artificial product, very much like what we now call alkali-albumin. The sole remnant of this theory now extant is the word 'Proteid.'

b. Schützenberger's theory.—Nasse[1] was the first who attempted to get an insight into the molecular constitution of the proteids by treating them in sealed tubes with baryta water at a high temperature for many hours. He found that the nitrogen was differently combined, part being easily displaceable and part held firmly. Schützenberger[2] has carried on researches in the same direction. He found that the products of decomposition are ammonia and carbonic acid in the same ratio as would result if urea were treated in the same way; other volatile products (pyrrole, indole, acetic acid, &c.), and a fixed residue in which the substances most abundantly present were leucine and tyrosine—the latter containing the aromatic radicle. Other substances also of the nature of amido-acids were found. These amido-acids he classifies into two groups—the leucines, or amido-acids of the acetic series ($C_nH_{n+1}NO_2$), and leuceines, or amido-acids of the acrylic series ($C_nH_{2n-1}NO_2$). Both leucines and leuceines are produced by the splitting up of bodies of the formula $C_mH_{2m}N_2O_4$ ($m=10$ or 12), which have a sweet taste and are therefore called gluco-proteins. Albumin is regarded as a ureide, or compound of urea; the urea is combined with gluco-proteins and the gluco-proteins split up on hydration into amido-acids. The nitrogen is thus after hydration combined as NH_2 (amidogen); in the proteid itself the nitrogen is probably present as NH (imidogen).

c. Pflüger's theory.—Although the distinction between living and non-living proteids was emphasised by John Fletcher[3] in 1837, it was not until 1875 that an intelligible theory to explain such difference was advanced by Pflüger.[4] The non-living proteids, such as are contained in white of egg, are stable and indifferent to neutral oxygen; but when these proteids are assimilated, that is, become part of a living cell, the molecules of proteid live by breathing oxygen; not necessarily oxygen from without, as frogs kept in chambers free from oxygen will continue to live for many hours. The assimilation of a proteid is probably due to the formation of ether-like combinations between the molecules of living proteid and the isomeric molecules of the food proteid, water being eliminated, this process of polymerism producing large and heavy but still simple molecules. In this process the nitrogen of the non-living proteid leaves the hydrogen with which it was combined in the form of amidogen (NH_2), and enters into combination with carbon to form the more unstable substance cyanogen (CN). We thus find uric acid, creatine, guanine, &c., as products of proteid metabolism, while none of such cyanogen-containing bodies are obtainable from non-living proteids.

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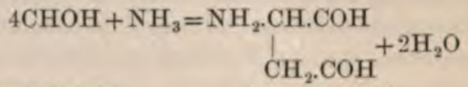
[1] *Pflüger's Archiv*, vi. 589.

[2] *Bull. Soc. Chim.* vols. xxiii. and xxiv.; *Annales de Chim. et Phys.* (5) xvi. 289; and a large number of papers in the *Compt. rend.* In a recent paper, *Compt. rend.* c. 1267, the formula for albumin given is simpler than those adopted in his earlier work; it is $C_{29}H_{48}N_8O_{10}$. More recently still (*C. R.* cvi. 1407) he has succeeded in preparing leuceine synthetically.

[3] *Rudiments of Physiology*, Edinburgh, 1837.

[4] *Pflüger's Archiv*, x. 251.

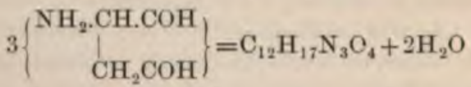
d. Loew's theory.—The researches of Loew and Bokorny[1] have taken the same direction as those of Pflüger, that is, they are attempts to explain the distinction between living and dead protoplasm. Living protoplasm or proteid in the cells of various algæ has the property of reducing silver from a weak alkaline solution of silver nitrate ; dead proteid has no such effect, and animal protoplasm is so quickly killed by silver nitrate that it also does not give the reaction. The conclusion arrived at is that something of the nature of an aldehyde occurs in living protoplasm. Formic aldehyde is probably formed in plants by the union of carbon and water ; if this is united to ammonia, aspartic aldehyde is formed, thus :—



$$4CHOH + NH_3 = \begin{array}{l} NH_2.CH.COH \\ \;\;\;\;\;\;\;\;| \\ CH_2.COH \end{array} + 2H_2O$$

[formic aldehyde] [aspartic aldehyde]

By polymerisation of aspartic aldehyde, we have—



$$3\left\{\begin{array}{l} NH_2.CH.COH \\ \;\;\;\;\;\;\;\;| \\ \;\;\;\;CH_2COH \end{array}\right\} = C_{12}H_{17}N_3O_4 + 2H_2O$$

and by further polymerisation in the presence of a sulphur compound and hydrogen, we get $6C_{12}H_{17}N_3O_4 + H_2S + 6H_2 = C_{72}H_{112}N_{18}SO_{22} + 2H_2O$, which represents the composition of ordinary albumin. The weak point of the theory is that the aldehyde of aspartic acid is unknown to chemists ; no doubt it is a most unstable substance. If such an aldehyde group does exist in living proteid, the instability of proteids is explicable, because molecular movements would be constantly occurring in the aldehyde group.

e. Latham's theory.—Latham[2] considers living proteid to be composed of a chain of cyanalcohols, or cyanhydrins as they are sometimes termed, united to a benzene nucleus.

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[1] *Loc. cit*

[2] *Brit. Med. Journ.* vol. i. 1886, p. 629.

Cyanalcohols are substances obtained by the union of an aldehyde with hydrocyanic acid, thus :—

$$\underset{\text{[ethyl alcohol]}}{C_2H_5.OH} + O = \underset{\text{[ethaldehyde]}}{CH_3.CHO} + H_2O$$

$$\underset{\text{[ethaldehyde]}}{CH_3.CHO} + \underset{\text{[hydrocyanic acid]}}{HCN} = \underset{\text{[cyanethylic alcohol]}}{CH_3CH(CN)OH}$$

Ethyl alcohol is taken as an instance in the above equations, but many other alcohols are considered to form similar cyan-derivatives, and these are united to one another and to benzene to form a proteid.

The theory is a satisfactory one, inasmuch as it includes the hypotheses both of Pflüger and Loew. Latham, moreover, shows exhaustively that the various products of the disintegration of albumin can also be obtained by the condensation and intramolecular changes that these cyanalcohols, which are exceedingly unstable bodies, undergo. Instability and proneness to undergo intramolecular changes are two properties common to living proteids and to cyanalcohols.

In an elaborate and painstaking manner Latham moreover adapts his theory to explain certain morbid processes ; he shows how, by a rearrangement of atoms different from that occurring in normal metabolism, excess of sugar may be produced in diabetes, excess of uric acid in gout, and certain ptomaines [1] in other complaints.

We can now leave these theoretical considerations and pass on to consider matters of greater practical interest.

TESTS FOR PROTEIDS

Solubilities.—All proteids are insoluble in alcohol and in ether. Some are soluble in water, others insoluble. Many of the latter are soluble in weak saline solutions. Some are insoluble, others soluble, in concentrated saline solutions. It is on these varying solubilities that proteids are classified.

All proteids are soluble with the aid of heat in concentrated mineral and acetic acids and caustic alkalis. Such treatment, however, decomposes as well as dissolves the proteid. Proteids are also soluble in gastric and pancreatic juices, but here again they undergo a change, being converted into a variety of proteids called peptones.

Heat-coagulation.—Many of the proteids which are soluble in water or saline solutions are rendered insoluble when those solutions are heated. The solidifying of white of egg under such circumstances is a familiar instance of heat-coagulation. Heat-coagulation must be very carefully distinguished from ferment coagulation—a process by means of which a ferment converts a previously soluble into an insoluble proteid; as instances of ferment coagulation the formation of fibrin in shed blood under the influence of fibrin-ferment, or of a curd of casein in milk under the influence of rennet, may be taken.

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[1] *Lancet*, vol. ii. 1888, p. 751.

The temperature at which a proteid enters into the condition of a heat-coagulum is fairly constant, and may be employed as one of the means of ascertaining what proteid is present in a given solution. The temperature varies somewhat with the reaction of the solution,[1] with the quantity and nature of the salts also present,[2] and, under certain circumstances, especially in an alkaline solution with the concentration of the solution.[3]

Unless a solution is very concentrated the contained proteid is not coagulated by heat in an alkaline solution, as it is converted into alkali-albumin; if the quantity of alkali is, however, very small, the temperature of heat-coagulation is raised. A neutral solution becomes alkaline after the separation of a heat-coagulum, and this alkalinity (produced no doubt by an alteration in the salts related to the proteid) may hinder the coagulation of the remaining proteid in the solution.

It is generally advisable to have the solution very faintly acid; a weak solution of acetic acid (2 per cent.) may be employed for the purpose of acidification. Acid-albumin does not form so readily as alkali-albumin, and the presence of a small amount of acid renders easier the separation of the coagulated proteid into flocculi, which can be then removed by filtration. An excess of acid lowers the temperature of coagulation, or it may convert the proteid into acid-albumin and so prevent coagulation altogether.

The simplest method of ascertaining the temperature of heat-coagulation is to place enough of the solution in a test-tube to cover the bulb of a thermometer. The test-tube, the contents of which should be kept constantly stirred by the thermometer, is then placed in a flask containing water and situated over a Bunsen burner. As the temperature rises the point at which flocculi separate should be carefully noted; a few degrees below this point the liquid becomes thick and opalescent. A form of double water-bath consisting of two beakers one within the other is recommended by Gamgee,[4] and Schäfer[5] has invented a very convenient form of running water-bath, the temperature of which can be easily changed (*see* fig. 44).

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[1] Halliburton, *Journ. of Physiol.* v. 165.

[2] Limbourg, *Zeit. physiol. Chem.* xiii. 450.

[3] Haycraft, *Brit. Med. Journ.* vol. i. 1890, p. 167.

[4] *Physiol. Chem.* p. 15.

[5] In my own work I have found certain inconveniences in the use of Gamgee's apparatus, and have therefore used Schäfer's. A description of it will be found in my paper in the *Journ. Physiol.* vol. v. p. 153.

Fractional heat-coagulation may be sometimes used for the separation of proteids from one another. Suppose one had a solution of fibrinogen and serum-globulin together, the solution faintly acidified is raised to the temperature of 56° C. and at that point the fibrinogen is precipitated; this is filtered off; the filtrate is once more raised to 56° in order to ascertain whether any fibrinogen remains in solution; if so, the heat-coagulum occurring at that temperature is once more filtered off and the filtrate again raised to 56°. When all the fibrinogen is removed, serum-globulin alone remains in solution, which is precipitated on raising the temperature to 75°. Serum-albumin [1] and egg-albumin [2] have also by this means been each differentiated into several proteids.

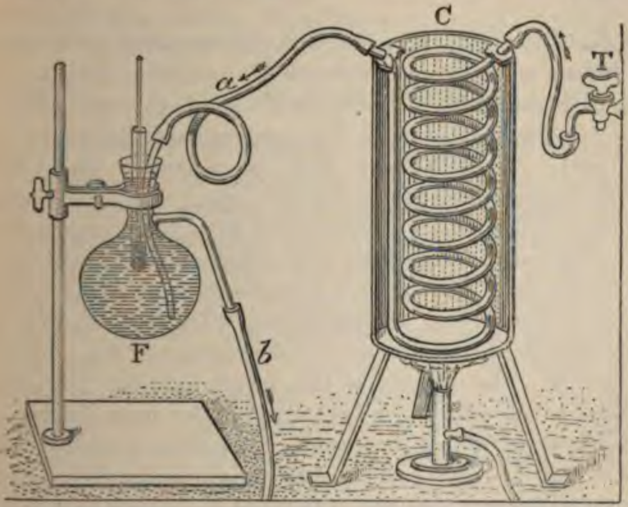

Fig. 44.—The liquid of which the heat-coagulation temperature is to be determined is placed in a test-tube in quantity sufficient to cover the thermometer bulb; this is placed in the neck of the flask F. Hot water enters the flask by the tube *a* and leaves by the tube *b*. The water comes from the tap T and is warmed by passing through the coil of tubing contained in a copper vessel filled with boiling water; the more slowly the water passes the hotter does it become; the rate of flow of water can be regulated by the tap. In the figure the front of the vessel C has been removed in order to show the coil of tubing within it.

The proteids which are coagulated by heating their solutions come under two classes—the *albumins*, which are soluble in water and in weak saline solutions, and the *globulins*, which are insoluble in water and soluble in weak saline solutions.

The temperatures of coagulation of some of the principal proteids are as follows:—

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[1] Halliburton, *Journ. of Physiol.* v. 159.

[2] Corin and Berard, *Travaux du laboratoire de Léon Fredericq*, Liège. ii. 170.

Albumins		*Globulins*	
Egg-albumin	73° C.	Fibrinogen	56° C.
Serum-albumin α	73°	Serum globulin	75°
" " β	77°	Cell-globulin	75°
" " γ	84°	Myosinogen	56°
Cell-albumin	73°	Myo-globulin	63°
Muscle-albumin	73°	Vitellin	75°
Lactalbumin	77°	Crystallin	73°
		Hæmocyanin	68°

Indiffusibility.—The proteids (with the exception of the peptones) belong to the class of substances called *colloids* by Thomas Graham. That is, they pass with difficulty, or not at all through animal membranes. In the construction of dialysers vegetable parchment is very largely used. Proteids may thus be separated from diffusible (*crystalloid*) substances like salts, but the process is a somewhat tedious one. The forms of dialyser used have been already described (p. 13). Take some serum, place it in a dialyser, and renew the water outside frequently. Some thymol crystals should be added to the serum to prevent the occurrence of putrefaction. The salts and extractions pass out through the membrane into the water, and the proteids alone remain within. The albumin is still in solution, but the globulin is precipitated as the salts which held it in solution have diffused out.

It is, however, found impossible even with the most prolonged dialysis to entirely remove all the salts which adhere to a proteid; so close is this adherence that one is inclined to believe that it is rather of the nature of loose chemical union. However carefully a proteid may have been purified, it always leaves on ignition a small quantity of ash, the composition of which varies in different cases, chlorides and phosphates of the alkaline metals and of calcium being the predominant constituents.

The term colloid does not necessarily imply that the substances in question are not crystallisable; for some of the vegetable proteids have been crystallised, and F. Hofmeister[1] states that by carefully evaporating a solution of pure egg-albumin half saturated with ammonium sulphate, he has succeeded in obtaining that substance in a crystalline condition.

Action on polarised light.—All the proteids are lævorotatory. If pure and in solution they may be identified and estimated by means of their specific action on polarised light (*see* p. 41).

The specific rotations (for the yellow line D) of some of the principal proteids are as follows:—

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[1] *Zeit. physiol. Chem.* xiv. 165.

Proteid	*Observer*	*Value of* $(\alpha)_D$
Serum-albumin	Hoppe-Seyler[1]	$-56°$
	Starke[2]	$-60°$
Egg-albumin	Hoppe-Seyler	$-33·5°$
	Haas,[3] Starke	$-38·08°$
Lactalbumin	Sebelien[4]	$-36°$ to $37°$
Serum-globulin	Haas	$-59·75°$
Fibrinogen	Herrmann[5]	$-43·0°$
Alkali-albumin	Haas	$-62·2°$
Syntonin (prepared from myosin)	Hoppe-Seyler	$-72°$
Casein (dissolved in $MgSO_4$ solution)	Hoppe-Seyler	$-80°$
Various albumoses	Kühne and Chittenden[6]	$-70°$ to $80°$

Colour reactions.—*a. Xanthoproteic reactions.*—Add a few drops of strong nitric acid; a white precipitate may or may not be produced according to the concentration and nature of the proteid. Peptones and certain varieties of albumose give no precipitate; other proteids do, unless the solution is very weak. Boil; the precipitate or liquid, as the case may be, turns yellow. Prolonged boiling always dissolves some of the precipitated proteid; those albumoses, which are precipitable by nitric acid, readily dissolve on heating. Cool; the liquid remains unaltered, except in the case of the albumoses; in their case, the precipitate reappears. Add ammonia, the yellow liquid or precipitate turns orange. It is this colouration which is the essential part of the reaction. It is the most delicate test for proteids we possess.

b. Millon's reaction.—One part by weight of mercury and two of strong nitric acid (sp. gr. 1·4) are mixed and gently warmed till the mercury is dissolved. The solution is diluted with twice its bulk of water, and the copious precipitate that forms is allowed to settle. The clear supernatant fluid is Millon's reagent. Add a few drops of this solution to a solution of proteid; a white precipitate is produced, which on heating becomes of a brick-red colour. This does not occur in the presence of sodium chloride. Millon's reagent precipitates many inorganic salts; but the precipitate of these does not turn red on boiling.

c. Adamkiewicz' reaction.—Add excess of glacial acetic and then concentrated sulphuric acid; a violet colour with feeble fluorescence is produced. This test is by no means a certain one, and is given by albumoses and peptones in concentrated solutions only.

d. Liebermann's reaction.[7]—If albumin is extracted with alcohol and washed with ether, it gives a deep violet colour when heated with concentrated hydrochloric acid.

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[1] *Zeit. f. Chem. u. Pharm.* 1864, p. 737.

[2] *Maly's Jahresb.* xi. 17.

[3] *Pflüger's Archiv*, xii. 378. *Chem. Centralbl.* 1876, 295, 811, 824.

[4] *Maly's Jahresb.* xv. 184.

[5] Herrmann, *Zeit. physiol. Chem.* xi. 508.

[6] *Zeit. Biol.* xx. 51.

[7] *Chem. Centralbl.* 1887, p. 600.

e. Piotrowski's reaction.—Add a few drops of a dilute solution of copper sulphate, a precipitate is produced of copper albuminate;[1] add excess of solution of caustic potash or soda, a violet solution is the result. If ammonia be used instead, a blue solution is the result.

In the case of the albumoses and peptones, however, the result is a rose-red solution with potash and a reddish-violet solution with ammonia. This is termed the *biuret reaction.* In performing this test great care must be taken to add very little copper sulphate; excess of copper sulphate gives a reddish-violet colour, which it is very difficult to distinguish from that given by ordinary proteids. This test is often performed in the presence of excess of neutral salts; in the case of magnesium sulphate, potash or soda gives a precipitate of magnesia which must be allowed to settle before the pink or violet solution can be seen; sodium chloride does not interfere with the reaction; when ammonium sulphate is present, a large excess of soda or potash must be added before the colour appears.

The term *biuret reaction* is given because the reddish-violet solution is very like that given under similar treatment by the substance called biuret. Biuret is formed from urea by heating it, ammonia is given off, and biuret remains:—

$$\underset{\text{[urea]}}{2CON_2H_4} - \underset{\text{[ammonia]}}{NH_3} = \underset{\text{[biuret]}}{C_2O_2N_3H_5}$$

Biuret yields on decomposition compounds containing cyanogen. For instance, by heat it is split into ammonia and cyanuric acid $(CN)_3H_3O_3$. Biuret, cyanuric acid, uric acid, xanthine, hypoxanthine, sarcosine, hydrocyanic acid, all give a similar reaction to the proteids. It is probable that the biuret reaction of proteids may be due to a cyanogen radicle.[2] In the above list, cyanuric acid most nearly resembles ordinary proteids (albumins and globulins) in the colours given, and peptones and albumoses give the same colours as hydrocyanic acid. The cyanogen in albumin and peptone is probably differently combined, corresponding to the similar differences in cyanuric and hydrocyanic acids respectively.

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[1] This preliminary precipitation is given by all proteids except deutero-albumose and peptone.

[2] Gnesda, *Proc. Roy. Soc.* 1890. Gnesda also gives a similar test in which nickel sulphate is employed instead of copper sulphate. Nickel sulphate and ammonia give no colour with albumins and globulins, a yellow colour with peptones and albumoses. Nickel sulphate and potash or soda give a yellow colour with albumins and globulins, an orange colour with albumoses and peptones. Here again the reactions are the same with cyanuric and hydrocyanic acids respectively.

Salkowski's Observations on the Colour Reactions of Proteids[1]

The following is an abstract of Salkowski's interesting observations on this subject :—

A part of the proteid molecule is aromatic ; and it is to the presence of aromatic radicles that the principal colour reactions of the proteids are due.

The aromatic substances derived from proteids on putrefaction fall into three groups :—

First group. The phenol group.—This includes tyrosine, the aromatic hydroxy-acids, phenol and cresol.

Second group. The phenyl group.—This includes phenyl-acetic and phenyl-propionic acids.

Third group. The indole group, of which indole, skatole, and skatole carboxylic acid are the most important members.

Whether all three groups exist preformed in the proteid molecule, or whether that molecule contains only one aromatic group, and the others are easily derived from this one on decomposition (Maly), matters little in a solution of the question investigated—namely, on which of the groups do the colour reactions depend? These reactions were tried with each of the substances enumerated, with the following results :—

i. *Millon's Reaction.*—Kühne considers that the reaction is due to tyrosine.[2] O. Nasse considers that it is due to those benzene derivatives in which only one atom of hydrogen is replaced by hydroxyl. Salkowski confirmed Kühne's observation. The reaction in question is given only by the substances in the first group just enumerated.

ii. *Xanthoproteic Reaction.*—This depends without doubt on the formation of nitro-derivatives, but the trinitro-albumin, and oxytrinitro-albumin of Löw are doubtful chemical units.

The substances of the first group give the reaction strongly.[3]

Those in the third group give it, but not so well.

Those in the second group do not give it at all.

Salkowski recommends that the intensity of the tint may be used to determine approximately the amount of peptone in a solution.

iii. *Adamkiewicz' Reaction.*—This is given only by substances in the third (indole) group, and especially by skatole carboxylic acid. The addition of a minimum quantity of potassium nitrite intensifies the colour, as it does also with a solution of proteids. A further addition of that salt turns the purple colour to red.

iv. *Liebermann's Reaction* is not given by any of the aromatic substances enumerated.

Precipitants of proteids. — Proteids are precipitated by a large number of reagents ; the peptones and albumoses are exceptions in many cases, but they will be considered separately afterwards.

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[1] *Zeit. physiol. Chem.* xii. 215.

[2] A striking confirmation of this view of Kühne's has been recently advanced by Kühne himself in conjunction with Chittenden (*Zeit. Biol.* xxii. 423), certain products of digestion are termed antiproducts. They yield on further treatment with digestive juices no leucine or tyrosine. On decomposing them with sulphuric acid, no tyrosine is obtainable. They also do not give Millon's reaction.

[3] In addition to these aromatic substances enumerated by Salkowski, leucine gives the reaction strongly.

Solutions of the proteids are precipitated by the following :—

1. Strong mineral acids, especially nitric, metaphosphoric,[1] and phosphotungstic acids.

2. Acetic acid and potassium ferrocyanide.

3. Acetic or oxalic acid, and excess of certain neutral salts like sodium sulphate, sodium chloride, or magnesium sulphate.

4. Salts of the heavy metals : basic lead acetate, mercuric chloride, silver nitrate, copper sulphate, ferric chloride or acetate, potassio-mercuric iodide, sodium tungstate, &c. The precipitates consist of the proteid in combination with the metal to form an albuminate. The rational formulæ for such compounds are however not known, and probably vary with different proportions of metallic salts and proteid. On the removal of the metal by a stream of sulphuretted hydrogen, the proteid is recoverable in an unchanged form.

5. By tannin ; or by tannin and sodium chloride together.

6. By saturation with ammonium sulphate, or sodio-magnesium sulphate, or potassium acetate, or potassium carbonate.

7. By picric acid. This test is often used for detecting albumin in urine.

8. By alcohol ; except in the presence of free alkali, when the proteids are slightly soluble in hot alcohol.

It is often of great importance to remove all the proteids which a liquid contains, so as to proceed to the detection of other substances.

The following are the best available methods :—

1. *Brucke's method.*[2]—This consists in acidulating the liquid with hydrochloric acid, and then adding to it a solution of potassio-mercuric iodide, made by saturating with mercuric iodide a boiling solution of potassic iodide.

2. *Girgensohn's method.*[3]—The solution is mixed with half its volume of a solution of common salt ; add tannin in slight excess, and the proteids are entirely precipitated.

3. *Hofmeister's method.*—To the liquid rendered faintly acid and heated to boiling, and from which all the proteids separable by mere boiling have been removed, a solution of ferric acetate, made by saturating acetic acid with recently precipitated ferric hydrate, is added. After boiling for a few minutes and filtering, a solution is obtained,

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[1] The precipitate with metaphosphoric acid resembles closely the phosphorised constituent of cell nuclei called nuclein (Liebermann, *Ber. d. deut. chem. Gesellsch.* xxi. 598).

[2] *Wiener Akad. Ber.* 1871.

[3] *N. Repert. Pharm.* xxii. 557.

which contains neither proteids nor iron. This method does not precipitate peptones.

4. *Wenz's method.*[1]—The solution is saturated with ammonium sulphate; all proteids but peptones are precipitated, and may be filtered off.

5. *By alcohol.*—If the solution is alkaline, it is rendered faintly acid with acetic acid, and several times its volume of absolute alcohol added. After twenty-four hours it is filtered; the filtrate is proteid-free.

6. *By boiling.*—In some cases the proteids are precipitable by simply boiling, after faintly acidulating the solution. This is the case if the proteids present belong to the albumin or globulin group, and such is usually the case with albuminous urine.

The words coagulation and precipitation are sometimes used synonymously, but in connection with the proteids, the two should be carefully distinguished. The term coagulation is used for the process that occurs when an insoluble proteid (coagulated proteid) is formed from a soluble one by the action of heat. This should be called *heat-coagulation*. The same word is used when a ferment like rennet causes the chief proteid in milk to become an insoluble curd; a similar ferment action occurs in blood-clotting. This should be termed *ferment-coagulation*. The term coagulation is also applied when a precipitate produced by the addition of a reagent to a solution of albumin is an insoluble one. Such precipitates are produced by the mineral acids, picric acid, tannin, and salts of the heavy metals. But there are other precipitants of proteids, in which the precipitated proteid is readily soluble in suitable reagents, and continues to show its typical characteristics. Such precipitation is not coagulation. Such precipitates are produced by saturation with various neutral salts, ammonium sulphate, sodio-magnesium sulphate, &c. It is possible that in these cases a compound of the proteid and the salt is formed, but an exceedingly loose compound.[2] Certain proteids (globulins) are more easily precipitated by this method than others. The globulins for instance are precipitated by saturating with magnesium sulphate or sodium chloride; these are salts, which do not precipitate albumins at all.

The precipitation produced by alcohol is peculiar, in that after a time it becomes a coagulation. Proteid freshly precipitated by alcohol is readily soluble in water, or saline media; but after it has been allowed to stand some weeks under alcohol, it becomes more and more insoluble. Albumins are the proteids which are most readily rendered insoluble by this method, then globulins; albumoses and peptones are apparently never rendered insoluble by the action of alcohol. This fact is of value in the separation of these proteids from others.

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[1] *Zeit. Biol.* xxii. 1.

[2] Other colloids possess the same property (gelatin, starch, &c.). Possibly in certain cases it depends on water-attracting power (Nasse, *Pflüger's Archiv*, xli. 504). For the action of a large number of neutral salts on proteids *see* Lewith, *Arch. exper. Path. und Pharm.* xxiv. 1. Hofmeister, *ibid.* 247. Halliburton, *Journ. Physiol.* v. 172.

QUANTITATIVE ESTIMATION OF TOTAL PROTEIDS

The methods that have been introduced to determine the total amount of proteids in solution are very numerous, and may be classified into gravimetric, or methods of weighing; densimetric, by estimation of specific gravity; and lastly methods depending on the quantity of nitrogen obtainable after combustion. The different methods for estimating particular kinds of proteids will be dealt with, when we speak of the proteids themselves in the tissues where they occur (*see* more particularly blood).

1. *Girgensohn's method.*—The precipitate produced by sodium chloride and tannin (*see* p. 124) is collected on a weighed filter, washed with water till free from salt, and then with boiling alcohol till free from tannin. It is dried at 110° and weighed. The amount of ash is ascertained after incineration and deducted. (In applying this method to urine, uric acid must first be separated by adding acetic acid and leaving the liquid in the cold some hours; then filtering off the uric acid crystals.)

2. *Precipitation by alcohol.*[1]—An accurately weighed or measured quantity of the solution is mixed with five times its volume of alcohol, and set aside for some hours; the precipitate is collected on a weighed filter, washed with hot alcohol and ether, dried, weighed, and the ash subsequently deducted. Schmidt[2] recommends the same method, but first neutralises the solution if necessary with acetic acid, and after twenty-four hours boils the liquid with the precipitate in it; it is then collected, washed, dried, and weighed as before.

3. *Precipitation by heat.*—The precipitate produced by boiling a known amount of a dilute solution of proteid, faintly acidified, may be collected, washed, and weighed as before: but this will only give accurate results, when the only proteids present are albumins and globulins.

4. *Densimetric method.*—Methods have been devised for the quantitative estimation of proteids in solution by means of multiplying the loss of specific gravity which such solutions undergo on removal of the

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[1] Hoppe-Seyler, *Handbuch*, p. 312.

[2] *Pflüger's Archiv*, xi. 10; *see* also Hoffmann, *Virchow's Archiv*, 1879, p. 255.

proteid by a constant factor, under the mistaken notion that the loss in density is directly proportional to the amount of proteid removed.[1] We have already seen that the method is a fallacious one in the case of dextrose (p. 97). The supposed constant factor is from its very nature a variable one, and a simple algebraical demonstration of this will be found in a paper by Huppert and Zahor.[2] Zahor[3] finds, however, that with urine it yields very good practical results. For clinical work, the specific gravity is estimated by a urinometer, marked to four places of decimals, before and after the separation of the albumin, by means of acidification (if necessary) and boiling. The approximate percentage of albumin is the loss of specific gravity multiplied by 400.

5. *Methods in which a nitrogen estimation is made.*—Ritthausen precipitates the proteids from solution with copper sulphate, collects the precipitate, and calculates the amount of proteid in it, by the amount of nitrogen obtainable in a combustion. Sebelien[4] has tested this method, using copper sulphate, lead acetate, phosphomolybdic acid, tannin and other precipitants. Tannin gave on the whole the best results. The nitrogen in the precipitate produced by tannin is estimated by Kjeldahl's method (*see* p. 23), and multiplied by 6·37 to obtain the total proteid. The method is stated to produce less error than the more usual methods involving the washing, drying, weighing, and incineration of proteid precipitates. A very similar method is adopted by König and Kisch,[5] who give the multiplier as 6·25.

CLASSIFICATION OF PROTEIDS

The proteids may be divided into animal proteids and vegetable proteids according to their origin. There appears to be no essential difference between these two classes, and each can be subdivided in the same manner into groups. The distinction, however, is a convenient one on which to form the basis of a classification.

A. Animal Proteids.—Class 1. *Albumins.*—These are proteids which are soluble in water, in dilute saline solutions, and in saturated solutions of sodium chloride and magnesium sulphate. They are, however, precipitated by saturating their solutions with ammonium sulphate. Their solutions are coagulated by heat, usually at 70°–73°C.

a. Serum-albumin. Not precipitated by ether.

b. Egg-albumin. Precipitated by ether.

c. Cell-albumin.

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[1] Bornhardt, *Zeit. Anal. Chem.* 1870, 149; 1877, 124.

[2] *Zeit. physiol. Chem.* xii. 467.

[3] *Ibid.* 484.

[4] *Ibid.* xiii. 135.

[5] *Zeit. Anal. Chem.* xxvii. 191.

d. Muscle-albumin.

e. Lactalbumin.

Class 2. *Globulins.*—These are proteids which are insoluble in water, soluble in dilute saline solutions, and insoluble in concentrated solutions of sodium chloride, magnesium sulphate, ammonium sulphate, and certain other neutral salts. Their solutions are precipitated by heat; the temperature of heat-coagulation varying considerably.

$\left.\begin{array}{l}\textit{a.}\text{ Fibrinogen.}\\ \textit{b.}\text{ Serum-globulin (paraglobulin)}\end{array}\right\}$ in blood plasma.

c. Globin; the proteid constituent of hæmoglobin.

d. Myosinogen, myoglobulin, &c., in muscle.

e. Crystallin; in the crystalline lens.

f. Vitellin; in yolk of egg, not precipitable by sodium chloride.

Class 3. *Albuminates or derived albumins.*—These are proteids derived from either albumins or globulins by the action of weak acids or alkalis. If a little solution of egg-albumin be warmed at 40°C. for 10–15 minutes with a few drops of 0·1 per cent. sulphuric acid, or 0·1 per cent. caustic potash, it will be found to have lost its typical properties, and to have been converted into acid-albumin or syntonin, and alkali-albumin respectively.

The albuminates are insoluble in pure water, and in neutral solutions containing no salt. They are soluble in acid or alkaline solutions, or in weak saline solutions. They are precipitated like globulins by saturation with neutral salts (sodium chloride, magnesium sulphate, ammonium sulphate). Their solutions are not coagulated by heat.

a. Syntonin or acid-albumin. Precipitated by neutralising its solution even in the presence of alkaline phosphates; the neutralisation precipitate dissolves in excess of alkali.

b. Alkali-albumin. Precipitated by neutralising its solutions. If alkaline phosphates are present, excess of acid must be added to cause precipitation, the alkaline phosphates being converted into acid phosphates, before the acid attacks the proteids. Alkali-albumin contains relatively less sulphur than syntonin. Some of the sulphur is removed by the alkali used to make alkali-albumin; what is left is more firmly combined and is not blackened by an alkaline lead solution. A very insoluble variety of alkali-albumin (probably a compound containing a large quantity of alkali) may be formed by adding strong potash to undiluted white of egg. The resulting jelly is called Lieberkühn's jelly.

c. Caseinogen. The chief proteid constituent of milk.

Class 4. *Proteoses.*—These are intermediate products in the hydration of proteids; the final products are called peptones. They are formed in the body by the action of the gastric and pancreatic juices; they may be also formed artificially by heating with water, or more readily by dilute mineral acids, or superheated steam.[1] They correspond to the propeptone of Schmidt-Mulheim, and to the A-peptone of Meissner. They have been chiefly worked at by Kühne and Chittenden, and will be more fully referred to in connection with digestion. They are not coagulated by heat; they are precipitated but not coagulated (*see* p. 125) by alcohol; they all give the biuret-reaction (rose-red colour with caustic potash and copper sulphate), and are precipitated by nitric acid, the precipitate being soluble on heating and reappearing when the liquid cools.

They may be sub-divided into albumoses, globuloses, vitelloses, caseoses, myosinoses, &c., according as the original proteid from which they are formed, is albumin, globulin, vitellin, casein, myosin, &c., respectively. The albumoses may be taken as an instance of the class. All the other groups may be subdivided in the same way.

The albumoses are of two varieties, *hemi-albumoses*, those which are converted by further digestive activity into hemipeptone, and *anti-albumoses*, those which are converted similarly into anti-peptone. According to their solubilities, albumoses are divided into :—

a. Proto-albumose. Soluble in cold and hot water and in saline solutions; precipitated like globulins by saturation with sodium chloride or magnesium sulphate.

b. Hetero-albumose. Insoluble in water; soluble in 0·5–15 per cent. sodium chloride solutions in the cold, but precipitated by heating to 65°. The precipitate is, however, not a heat-coagulum, as it readily dissolves in dilute acid or alkali. Hetero-albumose is precipitated by dialysing out the salt from its solutions. Like the other albumoses, it is precipitated by alcohol, but, unlike them, is partly converted into an insoluble product called dys-albumose. Hetero-albumose, like proto-albumose, is precipitated by saturation with salts. Proto- and hetero-albumoses are often called the primary albumoses, as they are the first products of the hydration of proteids.

c. Deutero-albumose. Soluble in cold and hot water. It is not precipitated from its solutions by saturating with sodium chloride or magnesium sulphate, but it is by ammonium sulphate. It is not precipitated by copper sulphate, and only gives the nitric acid reaction so characteristic of albumoses in the presence of excess of salt. It is thus in its reactions nearer to peptone, than the other albumoses ; it is an intermediate stage in the conversion of the primary albumoses into peptone.

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[1] Neumeister (*Zeit. Biol.* xxvi. 57) has recently found that the albumose formed by the action of superheated steam differs in a few minor reactions from those formed by acids or by gastric digestion. He has applied the name atmid-albumose to it.

Class 5. *Peptones.*[1]—These are the final products of the hydration of proteids. If hydration goes further the peptone is split into simpler substances and remains no longer a proteid. They are soluble in water, are not coagulated by heat, and are not precipitated by nitric acid, copper sulphate, ammonium sulphate, and a number of other precipitants of proteids. They are precipitated but not coagulated by alcohol. They are also completely precipitated by tannin, potassio-mercuric iodide, phosphomolybdic acid, phosphotungstic acid, and picric acid.

They give the biuret reaction (rose-red colour with a trace of copper sulphate and caustic potash or soda).

Pure peptone separated from all other proteids by ammonium sulphate, freed from excess of salt by dialysis, precipitated by alcohol and dried, hisses and froths with evolution of heat on being dissolved in water. Its taste is somewhat cheesy but not unpleasant. The bitter taste of artificially digested food is due to some product not yet separated, native proteids and albumoses being almost tasteless.

Peptones are divided into

a. Hemipeptone. The form of peptone which by the further action of pancreatic juice is split into simpler products, such as leucine and tyrosine.

b. Antipeptone. The form of peptone which is not decomposed in this way. It moreover yields no tyrosine on treatment with sulphuric acid, and does not give Millon's reaction.

Both forms of peptone are readily diffusible through animal membranes ; albumoses are only slightly diffusible. The utility of the formation of diffusible substances during digestion is obvious.

The table on the next page contrasts the chief reactions of the albumoses and peptone.

Class 6. *Coagulated Proteids.*—(*a*) Proteid in which coagulation has been produced by heat. Insoluble in water, weak acids, and alkalis. Soluble after prolonged boiling with concentrated mineral acids. Soluble in gastric and pancreatic juices giving rise to peptones.

(*b*) Proteids in which coagulation has been produced by ferments.

i. Fibrin. *See* blood.
ii. Myosin. *See* muscle.
iii. Casein. *See* milk.
iv. Anti-albumid. A comparatively insoluble by-product formed in gastric digestion.

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[1] See Kühne and Chittenden, *Zeit. Biol.* xxii. 423.

Variety of Proteid	Hot and Cold Water	Hot and Cold Saline Solutions e.g. 10% NaCl	Saturation with NaCl or $MgSO_4$	Saturation with Am_2SO_4	Nitric Acid	Copper Sulphate	Copper Sulphate and Caustic Potash
Proto-albumose	Soluble	Soluble	Precipitated	Precipitated	Precipitated in cold; precipitate dissolves with heat and reappears on cooling	Precipitated	Rose-red colour (Biuret reaction)
Hetero-albumose	Insoluble; i.e. precipitated by dialysis from saline solutions.	Soluble; partly precipitated, but not coagulated on heating to 65° C.	Precipitated	Precipitated	Ditto	Precipitated	Ditto
Deutero-albumose	Soluble	Soluble	Not precipitated	Precipitated	This reaction only occurs in presence of excess of salt	Not precipitated	Ditto
Peptone . . .	Soluble	Soluble	Not precipitated	Not precipitated	Not precipitated	Not precipitated	Ditto

B. Vegetable Proteids.—The amount of proteid matter in plants is less than in animals. Proteids occur either dissolved in the juices of plants, or in the solid form composing the protoplasm in the plant cells, or often deposited in the form of granules (aleurone grains). Vegetable proteids do not differ in their essential characteristics from animal proteids, but unlike most animal proteids they have frequently been obtained in a crystalline form.

Much error and confusion has crept into our knowledge of vegetable proteids from the researches of Ritthausen. He used caustic alkalis as a means of extracting the proteids from the vegetable tissues, and consequently converted the native proteids, globulin, albumin, &c., into alkali-albumin. It is necessary to remember that the substances legumin, conglutin, &c., which he thus obtained are artificial products, and do not represent what is present in the plant tissues themselves.

The vegetable proteids may be subdivided into the same six classes as the animal proteids.

Class 1. *Albumins.*—The term vegetable-albumin is often used synonymously with vegetable proteid; it should be properly restricted as in animals to those forms of proteid which are soluble in water and coagulable by heat. The greater part of the proteid coagulable by heat in the juices and seeds of plants is of the nature of globulin, not albumin. Small quantities of a true albumin have been described by Martin[1] in the juice of the papaw fruit, and by Green[2] in the latex of several caoutchouc-yielding plants of the natural orders *Apocyneæ* and *Sapotaceæ*.

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[1] *Journ. Physiol.* vi. 336.

[2] *Proc. Roy. Soc.* xl. 28.

Class 2. *Globulins*.—These are by far the most abundant proteids present in plants. This view, which has received the powerful support of Hoppe-Seyler,[1] who speaks of the proteids in buds, young shoots and seeds, as globulins, is contrary to that of Ritthausen, who on the ground of concordance in elementary analyses, regards vegetable proteids as consisting of legumin and other allied substances, which have been shown to be artificial products produced by the caustic alkali used in their preparation.[2]

The earliest observations of value on this subject are those of Weyl.[3] He compared the composition[4] and reactions of animal and vegetable proteids, he showed that the two classes were practically identical. He did not find any albumins, and examined the proteids extracted by salt solution from oats, maize, peas, mustard, Para nuts, &c., which consisted chiefly of plant-vitellin. A second proteid, also a globulin called plant myosin, was found in wheat, peas, oats, white mustard, and sweet almonds. No alkali-albumin (plant-casein or legumin of Ritthausen) was present in any of the plants examined.

The vitellin occurring in plants is often crystalline, and a number of observations have been made by different observers on this crystallised albumin, as it is often incorrectly called by them.

The proteids occurring in aleurone grains have been the subject of masterly researches by Vines[5]; he found much globulin there. The proteids of the papaw fruit, *Abrus precatorius*,[6] wheat and other flours[7] have been investigated by Martin; the change that occurs in the process of germination of seeds has been the subject of a research by Green[8] who has also investigated the proteids in latex. These researches may be briefly summarised as follows :—

Vines' Investigations on Aleurone Grains.—The aleurone grains of the peony (*Pæonia off.*) contain an albumose, and vegetable myosin; of the castor oil plant (*Ricinus comm.*) an albumose, a globulin insoluble in saturated sodium chloride solution (myosin), and a globulin soluble in that solution (vitellin); of blue lupin, chiefly crystalloid vitellin. Similar crystalloids were found in many other plants. The following classification of aleurone grains is given :—

1. Those soluble in water. Albumose.

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[1] *Physiol. Chem.* p. 75.

[2] Ritthausen defends his views in *Chem. Centr.* 1877, 567, 586.

[3] *Pflüger's Archiv*, xii. 635. *Zeit. physiol. Chem.* i. 72.

[4] For comparative elementary analyses, *see* A. Brittner, *N. Rep. Pharm.* xxi. 66 and 129.

[5] *Proc. Roy. Soc.* xxviii. 218; xxx. 387; xxxi. 62.

[6] *Ibid.* xlii. 331.

[7] *Brit. Med. Journ.* vol. ii. 1886, p. 104.

[8] *Proc. Roy. Soc.* xli. 446.

2. Those soluble in 10 per cent. NaCl.
    a. Grains without crystalloids. Soluble in saturated NaCl.
    b. Grains with crystalloids. Soluble in saturated NaCl.
3. Those partially soluble in 10 per cent. NaCl. Some of these are crystalloid, some insoluble, some soluble in saturated NaCl solutions.

Martin's Investigations on Papaw.—The proteids present are:—

1. A globulin very like serum-globulin.
2. Albumin. (Already alluded to, p. 131).
3. Albumoses of two kinds; with one of which (α-phytalbumose) a ferment (papain), in nature very like the trypsin of the pancreatic juice, is associated.

Martin's Observations on Wheat Flour.—The flour itself contains two proteids—vegetable myosin and an albumose. When mixed with water, these undergo certain changes, and are converted into the insoluble proteid called gluten.

Martin's Observations on Abrus (Jequirity).—Warden and Waddell[1] have given the name 'abrin' to the poisonous principle of jequirity; this plant is used as a drug to produce conjunctivitis when applied locally to the eye. It is not an alkaloid, but a proteid. The proteids are two in number—a globulin (resembling serum-globulin) and an albumose. Both proteids have a poisonous action, which is destroyed at a high temperature.[2]

Green's Investigations of Latex.—In Apocyneæ and Sapotaceæ the proteids are two albumoses and an albumin. In the manihot (Euphorbiaceæ) a globulin, in the common lettuce (Compositæ) an albumose.

We can now pass on to consider the chief members of the globulin group occurring in plants.

(a) Plant-vitellin (phyto-vitellin). This proteid is like animal vitellin, a globulin soluble in saturated solution of sodium chloride. It is coagulated by heat at about 75°C. In the yolk of the eggs of certain fishes, this substance has a semi-crystalline form, but in the aleurone grains of many plants it is distinctly crystalline, or can be made to crystallise. This is thus the purest proteid known, but even it leaves on ignition an ash consisting chiefly of alkaline phosphates. Elementary analysis gives C, 52·43; H, 7·12; N, 18·1; S, 0·55; O, 21·8 (Weyl).

The observations of Vines have shown that the crystalline proteids obtainable from aleurone grains differ in solubilities and crystalline form, and thus there is probably more than one crystallisable proteid.

The following are the chief observations made on the subject of crystalline vegetable proteids:—

Hartig,[3] in 1855, was the first to discover a crystalline proteid in plants.

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[1] *Non-bacillar Nature of Abrus Poison*, Calcutta, 1884.

[2] Martin, *Brit. Med. Journ.* vol. ii. 1889, p. 184.

[3] *Botan. Zeitung*, 1855, p. 881.

Maschke,[1] by extracting Para nuts with water at 50°, filtering and evaporating at the same temperature, obtained a crystalline proteid residue.

Weyl[2] identified the proteid as vitellin, and found it in a number of other plants.

Schmiedeberg[3] obtained a crystalline compound of this proteid with magnesia. Grübler[4] prepared the same compound and another, also crystalline, with lime; the molecular weight calculated from the first is 5081, that from the second 8848. Grübler's method of preparing the vitellin crystals is to dissolve the proteid from pumpkin seeds in solution of sodium chloride at 40°; on cooling the liquid to 7° the crystals (regular octahedra) separate. Ritthausen,[5] adopting the same method, obtained crystals (octahedra and rhombic dodecahedra) from expressed hemp cake, castor oil seeds, and the seeds of *sesamum indicum*.

Drechsel[6] introduced another method, which consisted in extracting the seeds (pumpkin) with water, and dialysing the extract into alcohol; the water diffuses into the alcohol, leaving crusts of microscopic crystals in the dialyser.

Vines found that the natural crystalloids imbedded in the ground substance of the aleurone grains were hexagonal rhombohedra in certain plants, and regular tetrahedra in others.

(*b*) Plant-myosin. This like animal myosin coagulates at 56°C. It also like the myosinogen of muscular tissue is converted into a more insoluble substance by a ferment action; this substance is called gluten-fibrin and forms the basis of gluten (*see* further, next page).

(*c*) Vegetable-paraglobulins.[7] This class of proteids coagulating at 75° and precipitated by saturation with sodium chloride was first described by Martin; one of these proteids occurs in papaw juice, another in latex, another in *abrus* seeds.

Class 3. *Albuminates.* — Acid-albumin or syntonin and alkali-albumin are formed readily by the action of acids and alkalis respectively on the native globulins of plants. Plant-myosin like animal myosin is especially readily convertible into these albuminates.

a. Legumin, or vegetable casein. The term legumin appears to have been used synonymously for vegetable proteid by the earlier investigators.[8] It has been the subject of laborious examination by Ritthausen.[9] We now know that it is simply alkali-albumin formed from the native globulins by the caustic potash used in extracting it from the plant.

b. Conglutin is the legumin obtainable from almonds and lupines. It is more glutinous and more soluble in acetic acid, and richer in nitrogen than ordinary legumin (Ritthausen).[10]

[1] *Journ. prakt. Chem.* lxxiv. 436.

[2] *Loc. cit.*

[3] *Zeit. physiol. Chem.* i. 205.

[4] *Journ. prakt. Chem.* cxxxi. 105.

[5] *Ibid.* p. 481.

[6] *Ibid.* (2) xix. 331.

[7] Martin, *Proc. Physiol. Soc.* 1887, p. 8.

[8] Einhof, *N. allgemein. J. d. Chem. v. A. Gehlen*, vi. (1805), pp. 126, 548. Dumas and Cahours, Liebig, and others, also examined this substance.

[9] *Zeit. f. Chem.* (2) iv. 528, 541; vi. 126; *J. pr. Chem.* ciii. 65, 78, 193, 273.

[10] *J. pr. Chem.* (2) xxvi. 440.

Class 4. *Proteoses.* — Previous to Vines's observations these were spoken of as vegetable peptones. Vines recognised that the aleurone grains did not contain true peptone, but a substance which he spoke of as hemi-albumose. Two albumoses of doubtful nature are described by Green in latex.

The following albumoses in plants have been more fully described (Martin):—

a. α-Phyt-albumose. Very like proto-albumose, and probably identical with Vines's hemi-albumose. Found in papaw juice, wheat-flour, *abrus.* Associated in papaw juice with the ferment papain.

b. β-Phyt-albumose. Very like hetero-albumose. Found in papaw juice.

c. Insoluble phyt-albumose. A constituent of gluten.

d. Vitelloses.[1] Intermediate products in the hydration of vitellin analogous to albumoses, and subdivided like them into anti-, hemi-, proto-, hetero-, dys-, and deutero-vitellose.

Class 5. *Peptones.*—True peptone does not appear to be found native in plants. It is formed from vegetable as from animal proteids by hydration processes, such as is brought about by boiling with dilute mineral acids, or treatment with gastric or pancreatic juices. The intermediate products are proteoses.

Papain like pancreatic juice acting on animal proteids converts them in an alkaline medium into proteoses, and finally peptone ; acting on vegetable proteids, it stops short at the proteoses, no true peptone being formed. Leucine and tyrosine are, however, found in the tissues of the plant (Martin).[2] Probably circulating proteid in the plant consists of albumoses.

Class 6. *Coagulated proteids.*—*a.* Proteids in which coagulation has been produced by heat. Albumin and globulin of vegetable origin, like the same substances of animal origin, are converted at a high temperature into an insoluble heat-coagulum.

b. Proteids in which coagulation has been produced by a ferment-action.

i. Gluten (the sticky constituent of dough which may be washed free from starch by kneading in a stream of water) is probably formed by a ferment-action from the proteids pre-existent in flour. This is supported by the fact that washing flour with water at a low temperature (2°C.) does not lead to the formation of gluten. The ferment has, however, not been separated.[3]

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[1] Neumeister, *Zeit. Biol.* xxiii. 402.

[2] *Journ. Physiol.* v. 213 ; vi. 336.

[3] Johannsen (*Ann. Agronom.* xiv. 420; *Abst. J. Chem. Soc.* 1889, p. 296) has advanced certain facts which tell against the ferment theory.

Boiling water or alcohol extracts from gluten a sticky substanc called insoluble phyt-albumose by Martin, and corresponding to tw substances, called gliadin and mucedin by Ritthausen.[1] The insolubl non-sticky residue is called gluten-fibrin.

We have already seen that the proteids in the flour itself are (1 vegetable-myosin, and (2) a soluble albumose. Probably they are th precursors of gluten, according to the following scheme (Martin):—

$$\text{Gluten} = \begin{cases} \text{Gluten-fibrin——precursor, myosin.} \\ \text{Insoluble albumose——precursor, soluble albumose.} \end{cases}$$

ii. Anti-albumid, anti-vitellid, &c., are substances of a compara tively insoluble nature formed during the earlier stages of gastri digestion.

Formation of and changes in the proteids in plants.—The formatio of proteids in plants is undoubtedly a synthetical process, the element and simple compounds, which are combined together to form them being ultimately derived from the soil and air. An exception to thi rule occurs in the carnivorous plants, and in parasitic plants, whic live upon the materials formed by other plants. In the carnivorou plants[2] (Drossera, Dionæa, &c.) a juice is secreted which has th power of converting into peptones the proteid matter in the flies an other small creatures caught by the plants.

Many nitrogenous bases are found in plants, such as asparagine leucine, tyrosine, adenine, &c. It is possible that these substances ar not always products of the breaking down of proteids, as they are i animals, but in certain cases, at any rate, are stages in the buildin up of the proteids.

Thus asparagine is probably formed by the union of inorgani nitrogen compounds with malic acid within the plant, the malic aci being derived from the carbohydrates,[3] which are formed by the unio of carbon and water under the influence of chlorophyll. But und certain other circumstances it has been shown that asparagine aris from the decomposition of proteids.[4] A large number of observations these nitrogenous bases, and the changes they undergo in germinatio have been made by E. Schulze,[5] but comparatively few on the chang that the proteids undergo. We have in plants certain reservoirs food material; this is especially seen in the cotyledons; this food material to be available for the needs of the plants must be converted into a soluble form. Starch, for instance, is converted into a soluble sugar, in most cases by the activity of an organised ferment, e.g. in malt; the proteids must be similarly changed, either into an albumose, peptone, or soluble nitrogenous base (asparagine, leucine, &c.), before it can be carried by the sap to other parts of the plant. Gorup-Besanez[1] stated that the changes in the reserve proteid materials during germination are probably due to the action of a ferment, and though this was disputed by Krauch,[2] subsequent experimenters are agreed that the ferment theory of Gorup-Besanez is probably correct. Green[3] considers that the nitrogen travels from the seed to the growing points in the form of amides, not in that of peptones or other proteids. Martin on the other hand is inclined to believe that the circulating nitrogen is chiefly contained in one or more soluble proteids of the albumose class.

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[1] *J. pr. Chemie*, lxxiv. 193, 384. For earlier observations on gluten, *see* Bouchar *Compt. rend.* xiv. 962; Taddei, *Giornale fisica di Brugnatelli*, xii. 360. *See* Günsberg, *J. pr. Chem.* lxxxv. 213.

[2] *See* Darwin, *Carnivorous Plants.* Article 'Carniv. Plants' in *Encyclop. E* (P. Geddes).

[3] Müller, *Landw. Versuchs. Stats.* 1886, 326.

[4] E. Schulze and E. Kisser, *Ibid.* xxxvi. 1.

[5] Numerous papers in *Zeit. physiol. Chem.* *See* especially xii. 405.

One of the best known ferments that effect these changes is papain or papayotin, obtainable from the juice of the papaw plant (Wurtz, Martin); but as the subject is more investigated, it becomes more and more strikingly demonstrated that papain is no single instance of a proteid-splitting ferment occurring in plant tissues, but that such ferments are practically ubiquitous.[4]

PROTEIDS AS POISONS

Albuminous substances form a most important element of food, but it is only within the last few years that the fact has been established that there are certain proteids which, when introduced into the circulation, are poisonous. This fact, remarkable as it is in itself, becomes of greater significance when it is considered that the poisonous proteids are not distinguishable by any well-marked chemical or physical properties from the non-poisonous or food proteids.

The most important of the vegetable proteid poisons are:—

1. Those contained in the seeds of jequirity, allusion to which has already been made (p. 133).
2. The proteid associated with or identical with papain.[5]
3. Lupino-toxin (?), from *lupinus luteus*.[6]

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[1] *Berichte deutsch. chem. Ges.* 1874, p. 1478.

[2] *Abst. Chem. Soc. Journ.* 1878, p. 996.

[3] *Proc. Roy. Soc.* xli. 466.

[4] *See* Thiselton Dyer's Presidential Address, Section D. *Brit. Assoc.* 1888 (Bath meeting); also Hansen, *Botan. Zeitung*, 1886, p. 137. Ellenberger and Hofmeister *Bied. Centr.* 1888, p. 319.

[5] Martin, *Brit. Med. Journ.* vol. ii. 1889, p. 184.

[6] *Schmidt's Jahrb.* 1888, cciv. 10.

The most important of the animal proteid poisons are :—

1. Snake poison. Bacteria and alkaloids are here absent,[1] and the proteids obtained in a pure condition are as poisonous as the original venom. Fayrer, Brunton, Weir-Mitchell, Wolfenden, Reichardt, and others, are unanimous on this point. The poison has been examined in the cobra, viper, crotalus, copperhead, mocassin, and the same result arrived at in all cases.

Wolfenden,[2] who has examined the venom of the cobra and viper according to the most recent methods of separating proteids, finds in the former (1) globulin, (2) albumin, and (3) syntonin ; and in the latter (1) globulin, (2) albumin, and (3) an albumose. All these are poisonous. The chief symptom produced is asphyxia.

2. The proteids in the serum of certain fishes (conger eel, muræna, &c.).[3]

3. Proteid poisons found in certain spiders.[4]

4. Proteids formed during natural digestion in the stomach and small intestines—albumoses and peptones.

5. Wooldridge's tissue-fibrinogens which produce intravascular coagulation of the blood.

6. Fibrin-ferment.

The poisonous proteids numbered 4, 5, and 6 will be more fully discussed in connection with the blood.

Poisonous proteids produced by bacterial activity will be referred to under Fermentation (Chaps. XII and XIII).

In the following table Martin[5] compares the activity of some of the most important proteid poisons :—

	Fatal Dose
Venom of common adder[6]	0·0021 gramme per kilo of body weight
Venom of Australian tiger snake[7]	0·0049 ,, ,,
Venom of cobra[8]	0·000079 ,, ,,
Abrus poison :—	
Globulin	0·01 ,, ,,
Albumose	0·06 ,, ,,
Peptic albumoses[9]	0·3 ,, ,,

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[1] In some cases alkaloids are present, but they are non-poisonous ones (Gautier).

[2] *Journ. Physiol.* vii. 327. References to other writers will be found here; the cobric acid of Blyth (*Analyst*, i. 204) is shown to be non-existent.

[3] Mosso, *Maly's Jahresbericht*, xviii. 92.

[4] Kobert, *Sitzungsb. der Dorpater Naturforsch. Gesell.* 1888; abstracted in *Centralbl. f. d. med. Wiss.* 1888, p. 544.

[5] *Proc. Roy. Soc.* xlvi. 108.

[6] Fontana, quoted in *Marx. Giftlehre*, ii. 74.

[7] Report of Special Commission on Snake Poisoning, *Austral. Med. Journ.* 1876, No. 21, p. 104.

[8] Vincent-Richards, *Landmarks of Snake-poison Literature.*

[9] Pollitzer, *Journ. of Physiol.* 1886.

TABLES ILLUSTRATING METHODS OF TESTING FOR PROTEIDS

The following tables present in a compact form the chief analytical methods of separating and identifying the most important proteids when in solution :—

TABLE I.—*One proteid only present*

a. With small portions of the solution ascertain the presence of a proteid by the xanthoproteic, Millon's, and other tests.

b. Determine the reaction of the solution.

If acid	*If alkaline*	*If neutral*
Test for acid-albumin. This does not coagulate on boiling, and gives a precipitate on neutralisation, dissolving again in excess of alkali.	Test for alkali-albumin. This does not coagulate on boiling, and gives a precipitate on neutralisation, dissolving in excess of acid. If caseinogen is present test with rennet—*see* milk.	Acid and alkali-albumin must be absent, unless neutral salt is present. They do not coagulate on boiling, and are precipitated by saturation with $MgSO_4$ like globulins. For caseinogen *see* milk.

c. Faintly acidify (if necessary) and boil. There may be a heat-coagulum or there may not.

If the proteid is coagulated by heat. The proteid is then either an

ALBUMIN or GLOBULIN,

which may be identified as follows with fresh portions of the solution :—

ALBUMIN	GLOBULIN
1. Gives no precipitate on saturation with $MgSO_4$.	1. Is precipitated by saturation with $MgSO_4$.
2. Ascertain temperature of heat-coagulation.	2. Ascertain temperature of heat-coagulation.
3. Serum-albumin is not, egg-albumin is, precipitated by ether.	3. Certain globulins (fibrinogen, myosinogen, &c.) behave in a characteristic manner to certain ferments.

If the proteid is not coagulated by heat. The proteid is then either

ALBUMINATE	ALBUMOSE	PEPTONE
Acid or alkali albumin, or caseinogen. See *b*.	1. Biuret reaction. 2. Characteristic reaction with nitric acid. 3. Saturate with NaCl or $MgSO_4$.	1. Biuret reaction. 2. No precipitate with nitric acid. 3. No precipitate on saturation with Am_2SO_4. *All other proteids are precipitated by saturation with Am_2SO_4.*

ALBUMOSE (continued):

If a precipitate falls a primary albumose is present. Submit the solution to dialysis.		No precipitate.
No precipitate occurs, proto-albumose is present.	A precipitate falls. Hetero-albumose is present. This is also precipitated by warming to 65°, which precipitate is soluble in weak acid or alkali.	Deutero-albumose is present; this gives no precipitate with $CuSO_4$. Gives characteristic nitric acid reaction only in presence of excess of salt. Gives a precipitate by saturating with Am_2SO_4.

TABLE II.—*Separation of proteids when more than one is in solution; albumoses and peptones being absent*

a. If the solution is acid or alkaline test for acid-albumin and alkali-albumin; if present, neutralise and filter off the precipitated proteid. (N.B.—Weak solutions of globulins are sometimes precipitated by a small quantity of dilute acid—*see* serum-casein; to avoid error, it is therefore best to proceed also as directed under *c*, next page.)

b. Neutralise solution if necessary (acid- or alkali-albumin having been removed). Saturate with magnesium sulphate. (Sodium chloride produces a less complete precipitation of many proteids.)

The precipitate consists of GLOBULINS.

Wash precipitate with saturated solution of $MgSO_4$, and dissolve by adding water; the salt adherent to the proteid renders it soluble. Remove excess of salt by dialysis.

i. Add fibrin ferment.	ii. Add myosin ferment.
Fibrinogen is converted into fibrin.	Myosinogen is converted into myosin.

iii. Heat another portion to 60°. Fibrinogen and myosinogen are precipitated; filter. The filtrate contains:—

Myoglobulin	*Paraglobulin*	*Cell-globulin*
Precipitated at 63°.	Has no fibrinoplastic action.	Possesses fibrinoplastic action.
	Precipitated at 75°.	

The filtrate contains the

ALBUMINS

Dialyse away excess of salt. Saturate with ammonium sulphate; wash the precipitated albumins with saturated solution of ammonium sulphate; dissolve the precipitate by adding water; excess of salt may again be removed, if necessary, by dialysis. A solution of pure albumin is thus obtained. Add ether, egg-albumin is precipitated, serum-albumin remains in solution.

VITELLIN (a globulin), which is very imperfectly precipitated by salt, may be mixed with the albumins. If so, it will be precipitated by dialysis. (For crystalline vitellin *see* p. 133.)

c. Slightly acidify the solution and boil; the albumin and globulin are coagulated; filter off the precipitate, and again test for acid-albumin and alkali-albumin. See *a.*

TABLE III.—*To separate the coagulable proteids (albumins and globulins) from the non-coagulable (albumoses and peptones)*

a. Saturate with ammonium sulphate. All proteids are precipitated but peptones; filter off the precipitate; the peptones are in the filtrate.

b. Boil (after acidification). This precipitates the albumins and globulins; filter. The filtrate contains the albumoses and peptones, which may be separated as in Table IV. This method is only applicable to concentrated solutions, as small quantities of primary albumoses are apt to be formed by the hydrating action of the acidified hot water from the albumins and globulins.

c. Add ten times its volume of alcohol to the solution. This precipitates all the proteids. Leave the precipitate under absolute alcohol for at least one—better two or three months. Pour off supernatant alcohol, evaporate the rest of the alcohol at 40° C. Add water. The albumoses (except dys-albumose) and peptones enter into solution. Separate as in Table IV. Albumins and globulins are, as a result of the prolonged action of alcohol, insoluble in water or saline solutions.

d. Saturate with magnesium sulphate. A precipitate is produced; filter this off.

The precipitate contains globulins, proto-albumose, and hetero-albumose. Separate these as in *b* or *c*.	The filtrate contains albumin, vitellin, deutero-albumose, and peptone. Separate as in *b* and *c*. For the separation of vitellin, precipitate it by dialysis as in Table II.

TABLE IV.—*Separation of proteids when more than one is in solution; globulins, albumins, and albuminates being absent.*

<table>
<tr><td colspan="3">The solution will contain proteoses (albumoses) and peptones. Faintly acidify with acetic acid, and fully saturate with ammonium sulphate. A precipitate is produced, filter this off.</td></tr>
<tr><td colspan="2">The precipitate consists of
ALBUMOSES
Wash the precipitate with saturated solution of Am_2SO_4. Redissolve the precipitate by adding water. Render the solution faintly acid with acetic acid. Saturate with sodium chloride, and filter off the precipitate.</td><td rowspan="2">The filtrate contains the
PEPTONES
To separate these in a pure state, evaporate the solution to a small bulk, filter off the crystals of ammonium sulphate which separate, and the remainder of the salt by aqueous baryta, and the last traces by barium carbonate. Precipitate the excess of baryta by dilute sulphuric acid; filter off the barium sulphate thus precipitated. To the filtrate add excess of alcohol; this precipitates the peptones; redissolve them in a small quantity of water, and reprecipitate by phospho-tungstic acid; wash the precipitate with alcohol and ether, and dry.</td></tr>
<tr><td>The precipitate consists of <i>primary albumoses.</i> Wash the precipitate with saturated solution of NaCl, and redissolve it by adding water. Dialyse the solution.
<i>Hetero-albumose</i> is precipitated; the precipitate is collected, washed, and dried; or may be first redissolved and then precipitated by alcohol.
<i>Proto-albumose</i> remains in solution and may be precipitated by alcohol, washed, and dried.</td><td>The filtrate contains <i>deutero-albumose,</i> which may be precipitated by saturation with ammonium sulphate or evaporated to a small bulk and precipitated by alcohol, washed with alcohol and ether, and dried.</td></tr>
</table>

CHAPTER XI

THE ALBUMINOIDS, FERMENTS, AND PIGMENTS

THE ALBUMINOIDS

THE term albuminoid is used by some chemists synonymously with proteid. It is however best to restrict the name to a group of substances which, although similar to the proteids in many particulars, differ from them in certain other points. No doubt in most cases they originate from proteids. They are especially abundant in the connective tissues and in epithelium, and they will be fully described in connection with these tissues.

1. *Collagen.*—The substance of which the white fibres of connective tissue are composed. It is probably the anhydride of gelatin.

2. *Ossein.*—The collagen from bone.

3. *Gelatin.*—The substance produced by boiling collagen with water. Soluble in hot, insoluble in cold water. It is not precipitated by acetic acid and ferrocyanide of potassium. It contains no sulphur. $(a)_D = -130°$.

By hydrating agents, such as heating with superheated steam, treatment with gastric juice, &c., it is converted into peptone-like substances, intermediate bodies analogous to the proteoses being formed. Salkowski[1] gives the following differences between proteid-peptone, gelatin, and gelatin-peptone.

	Proteid-peptone	*Gelatin*	*Gelatin-Peptone*
Adamkiewicz' reaction	violet	yellowish	yellowish
Addition of an equal volume of concentrated H_2SO_4	dark brown	yellow	yellow
Millon's reaction	reddish pp.	colourless	colourless
Xanthoproteic reaction	deep orange	lemon-yellow	lemon-yellow

4. *Chondrigen.*—The organic basis of hyaline cartilage; it is a mixture of collagen and mucinoid substances.

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[1] *Berlin. klin. Woch.* 1885, No. 2.

5. *Chondrin.*—The substance obtainable from chondrigen by boiling. It is a mixture of gelatin and mucinoid substances.

6. *Mucin.*—A widely distributed substance occurring in epithelial structures (mucus, mucous glands, goblet cells, cement-substance of epithelium), in connective tissues (chief constituent of the ground-substance); it forms the chief constituent of the bodies of certain invertebrates like the snail; it is found in electrical organs, in synovia, in certain forms of saliva, and in bile. The greater part of the slimy substance in bile is however a nucleo-albumin.

The mucin obtainable from different sources varies in composition and reactions. There are probably several mucins. They all agree in the following points.

(*a*) Physical character. Viscid, slimy, tenacious.

(*b*) Precipitability from solutions by acetic acid; they are insoluble in excess of this reagent. They all dissolve in dilute alkalis.

(*c*) They are all glucosides: compounds of a proteid (probably variable, but generally a globulin) with animal gum, which by treatment with dilute sulphuric acid can be hydrated into a reducing, but non-fermentable sugar.

7. *Colloid-substance.*—This occurs in the thyroid gland, and in certain forms of tumour, especially those connected with the thyroid (goître) or with the ovary (*see* ovarian cysts). Acetic acid causes it to swell, but does not precipitate it, otherwise it resembles mucin.

8. *Met-albumin or pseudo-mucin* is the same as colloid substance (*see* ovarian fluid).

9. *Paralbumin* is met-albumin in loose combination with proteid substance.

10. *Lardacein.*—This substance occurs in that form of degeneration called waxy or albuminoid degeneration. It specially affects small blood vessels, but it may also involve the tissue-elements of organs like the liver, spleen, pancreas, &c. This form of degeneration occurs especially in cases of chronic pus-formation. The parts affected become brownish-red, as glycogen does on the addition of iodine, and bluish or violet on the addition of sulphuric acid and iodine. It was therefore supposed at one time to be of the nature of a carbohydrate, and was called amyloid substance by Virchow. Kekule[1] has, however, shown that it is nitrogenous, and is probably an intermediate step between albuminous matter on the one hand and fat and cholesterin on the other. It is insoluble in water, alcohol and ether. Dilute acetic acid has no effect on it, except that the strong acid causes it to swell. It is insoluble

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[1] Kekule and Friedreich, *Virchow's Archiv*, xvi. 58. *See* also Kühne, *Maly's Jahresb.* iii. 31.

in alkaline carbonates, but dissolves in strong alkalis. Until quite recently it was also stated to be insoluble in gastric juice; but although it is acted upon with difficulty, gastric juice does ultimately dissolve it (Kostiurina).[1]

11. *Elastin.*—This is the substance of which the yellow fibres of connective tissue are composed. It is a very insoluble material. The sarcolemma of muscular fibres and certain basement membranes are very similar.[2]

On digestion, elastoses (substances analogous to the proteoses) are formed.[3]

12. *Nuclein.*—The chief constituent of cell-nuclei. A similar substance is also found in milk, and yolk of egg. Its physical characters are somewhat like mucin; it however contains abundance of phosphorus.

A substance very similar to nuclein has been made artificially by adding metaphosphoric acid to albumin. Nuclein and the chromatin of histologists are probably identical. (*See* The Nucleus, Chap. XIV).

13. *Plastin.*—Another highly phosphorised substance found in nuclei and cell-protoplasm.

14. *Nucleo-albumins.*—Compounds of proteids (generally globulins) with nuclein.

These are constituents of cell protoplasm, and are perhaps identical with the plastin of microscopists. The mucin-like substance in bile is a nucleo-albumin.

15. *Spermatin* is the mucin-like substance in semen. It however differs from mucin in being soluble in excess of acetic acid. Possibly it also may be a nucleo-albumin.

16. *Hyalins and Hyalogens.*—A group of substances very like the mucins, chiefly found in invertebrate connective tissues (Krukenberg. *See* Chapter XXII).

17. *Keratin.*—Horny material. *See* Chapter XXI.

18. *Eleidin.*—A stage in the formation of keratin.

19. *Skeletins.*—A name given by Krukenberg to a number of insoluble epithelial products found chiefly in invertebrates. The group includes *chitin, conchiolin, cornein, spongin, fibroin, and silk* (*See* Chapter XXI).

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[1] *Chem. Centralbl.* 1887, p. 120.

[2] Certain minor differences have been recently pointed out by Ewald, *Zeit. Biol.* xxvi. 1.

[3] Horbaczewski, *Zeit. physiol. Chem.* vi. 330. Chittenden and Hart, *Zeit. Biol.* xxv. 368.

THE FERMENTS

The ferments are substances which produce chemical changes in other substances, without apparently undergoing any change, or at least without forming any constituent part of the final products.

The ferments are divided into two great groups.

1. The organised ferments : that is to say, living organisms. Yeast and bacteria may be cited as instances.

2. Enzymes or unorganised ferments. Chemical principles excreted either by organised ferments, or the product of the activity of other living cells, e.g. those of the glands of the stomach, pancreas, &c. The different methods in which such ferments may originate within cells are briefly described under 'secreting epithelium' (Chap. XXI).

The ferments, whether they consist of living organisms or not, are exceedingly unstable substances, and thus resemble what we know is the case in all protoplasm which is living, especially in the proteid constituents of protoplasm. It is no doubt this very instability, and the intramolecular changes with which it is associated, that confer on ferments their special power of producing molecular rearrangements of the substances with which they come in contact.

The actual ferments are substances which elude the grasp of the investigator to a great extent. They however appear to be either proteids, or substances nearly related to the proteids. In the case of certain ferments, however, it has been actually demonstrated that they are proteids, e.g. fibrin-ferment,[1] pepsin,[2] and malt diastase.[3] In the case of diastase Loew has also demonstrated the interesting fact that, like a living proteid, it contains an aldehyde radicle. Schützenberger's[4] analysis of yeast shows that this substance yields in addition to inorganic salts and gum (arabin), a number of amido-acids (leucine, tyrosine, guanine, &c.), such as are always obtainable from proteids.

THE PIGMENTS

The pigments form a class of substances of the greatest importance. The purely chemical pigments have been the subject of painstaking research, and this has been followed, especially in the case of the aniline dyes, with important industrial results.

But the pigments occurring in nature have received, if not scant attention, at any rate a form of attention that has not resulted in intimate acquaintance, and in many cases the field is still a blank.

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[1] Halliburton, *Journ. Physiol.* ix. 229.

[2] Langley and Edkins, *Ibid.* vii. 371.

[3] O. Loew, *J. pr. Chem.* (2) xxxvii. 101.

[4] *Compt. rend.* lxxviii. 493.

The chemical constitution of the ordinary vegetable dyes, of which logwood is a familiar example, is unknown. Still more is our ignorance apparent when such substances are associated with proteids, as in many of the most important of the pigments both in plants (chlorophyll), and in animals (hæmoglobin).

There are vast fields of research—such, for instance, as the pigments of feathers, of skins, eggs, butterflies' wings, flowers, &c.—of which the fringe is only just touched, but the results are so meagre that it will be no benefit to mention the few disjointed facts that have been ascertained.

The function of pigments is also most important: in certain cases the pigment is attractive; in certain others protective; in others, again, it is of service in the functions of respiration, vision, &c.

The increasing use of the spectroscope in the investigation of the natural pigments will no doubt in time be followed by results similar to those which followed the invention of the microscope in anatomy, or of the telescope in astronomy.

Bearing in mind our present deficiencies, the following groups of pigments may be mentioned:—

1. *The Respiratory or Proteid Pigments.*—These pigments are either proteids or associated with proteids. Some perform the function of carriers of oxygen; receiving it from the air, and carrying it to the tissues. These are the pigments of the blood (hæmoglobin, hæmocyanin, &c.). Some perform the function of receiving the oxygen from the blood, and holding it until the tissue elements are in need of it. These are the histohæmatins. Others, again, have relation to the carbonic acid; these are the chromophylls, the most important of which is chlorophyll; it occurs in the majority of plants, and in a few animals.

(*a*) Hæmoglobin. The red pigment of the blood is remarkable for containing iron, for being associated with a proteid, and therefore non-diffusible, but yet is crystalline.

(*b*) Hæmocyanin (a blue pigment containing copper), chlorocruorin (a green pigment containing iron), and others, take the place of hæmoglobin in many invertebrates.

(*c*) The histohæmatins. These also contain iron. Myohæmatin, the most important member of the group, occurs in muscle, and will be found fully described in Chap. XX.

(*d*) Chlorophyll; *see* end of Chap. XIV.

2. *The derivatives of Hæmoglobin.*—Many derivatives of hæmoglobin can be artificially produced by the action of reagents; some of these, e.g. methæmoglobin, hæmatoporphyrin, &c., sometimes occur in the

body as the result of similar decompositions to those produced in th laboratory. Others, e.g. hæmin, are never found in the body.

The pigments occurring in other parts of the body are mostl derived from hæmoglobin.

Thus the pigments of the bile (bilirubin, &c.), of the urine (urobilin, &c.), of the fæces (stercobilin, &c.), undoubtedly take their ultimate origin from hæmoglobin, and it is possible that the source of the histohæmatins is the same.

The pigments of the bile, urine, and fæces have however no special function, and appear to be merely the channels for the excretion of 'effete' blood pigment.

3. *The Lipochromes.*—These are the fatty pigments. The name lutein (Thudichum[1]) was at one time given to the group. They are exceedingly numerous. The chief members of the group are as follows :—

(1) The yellow pigment of the corpus luteum : lutein.

(2) The yellow pigment of fat, butter, yolk of egg, and blood-serum.

(3) Carrotin, the colouring matter of the carrot and tomato, is a lipochrome which has been more fully examined than many of the others. It, like all the rest, consists of carbon, hydrogen, and oxygen. Its formula is $C_{18}H_{24}O$ (Husemann[2]).

(4) Tetronerythrin. The name was first given by Wurm[3] to the red pigment round the eyes of certain birds. Merejkowski[4] has since found it in 104 species of animals, both vertebrate and invertebrate. It is the red pigment occurring in the shell, blood, and hypoderm of the lobster and allied crustacea. It has been the subject of study by MacMunn,[5] myself,[6] and others. It probably has no such respiratory activity as Merejkowski imagines, and probably the word as used by Merejkowski may include several distinct reddish lipochromes.

(5) The chromophanes. The pigments of the retinal cones.

(6) Visual purple ; the pigment of the retinal rods is either a lipochrome or closely allied to one.

(7) Xanthophyll and other yellow pigments occurring in leaves, flowers, and fruit.

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[1] *Centralbl. med. Wiss.* vii. 1.

[2] Husemann, *Liebig's Annalen*, cxvii. 200. Arnaud, *Compt. rend.* cii. 1119; civ 1293, gives the formula $C_{26}H_{38}$, but as no other coloured hydrocarbon is known, carrotin probably contains oxygen.

[3] *Zeit. wiss. Zool.* xxxi. 535.

[4] *Compt. rend.* xciii. 1029.

[5] *Proc. Birmingham Philosoph. Soc.* iii. 351. *Proc. Roy. Soc.* 1883, p. 17.

[6] *Journ. Physiol.* vi. 324.

The lipochromes are characterised :—

(1) By solubilities in ether, alcohol, benzene, turpentine, &c., like the fats.

(2) By certain colour reactions : a green or blue colour with iodine, or with sulphuric acid, or with these two reagents together ; a green colour with nitric acid.

(3) By absorption bands towards the violet end of the spectrum.

(4) By being bleached (after varying periods) by light. For the bearing of this on the subject of vision *see* Chap. XXI.

4. *The Melanins.*—The black pigments of the body are numerous; their origin is doubtful ; some, like Lehmann,[1] looking upon them as derivatives of hæmoglobin, and others, e.g. Nencki, regard them as free from iron and containing sulphur, so that they cannot be derivatives of hæmatin. Some, e.g. Krukenberg, regard them as nitrogenous derivatives of lipochromes. It is probable that there are several melanins, that some contain iron, and some do not. They are not humous substances (Hirschfeld[2]). They will be found described in connection with the tissues where they occur.

(*a*) Fuscin, the black pigment of the retina.

(*b*) The black pigment of the skin and hair.

(*c*) The black pigment of melanotic sarcomata.

(*d*) Other black pigments in different parts of the animal kingdom, e.g. those described by Krukenberg in the stems of Gorgonidæ and the shells of mollusca.

5. *Indigo pigments.*—*See* p. 78.

6. *Humous substances.*—*See* p. 95. These as a rule are not nitrogenous and of carbohydrate origin, but certain members of the group are stated by Udránszky[3] to be nitrogenous ; and the dark colour that occurs in treating urine with mineral acids is considered by him to be due to such a humous substance formed from the small amount of carbohydrate present normally in urine and urea. The dark colour of horses' urine is stated to be chiefly due to a humous substance formed from some constituent of the fodder ; as is also the dark colour of the urine after the administration of carbolic acid.

7. *Miscellaneous pigments.*

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[1] *Handbuch physiol. Chem.* p. 166.

[2] *Zeit. physiol. Chem.* xiii. 407.

[3] *Ibid.* xi. 587 ; xii. 33. This pigment is probably that described by many observers under different names (Proust's fallow resin, Scharling's omichmyl oxide, Heller's urrhodine, Schunck's indirubin, Scherer's pigment from urine, Harley's urohæmatin, Marcet's immediate principle, Thudichum's uropithin, uromelanin and omicholic acid, Heller's urophæin, and several others).

occurs only when the organisms are growing, and stops when the organisms are removed or killed.

This vitalistic theory of fermentation becomes especially important to the physiologist and pathologist when applied to disease. The 'germ theory,' as it is termed, explains the infectious or zymotic diseases by considering that the change in the system is of the nature of fermentation, and like the other fermentations we have mentioned, produced by particular forms of bacterium; the transference of the bacteria or their spores from one person to another constituting infection. This theory has not been fully verified for every infectious disease by the discovery of a specific microbe; many able investigators, however, consider it likely that the pathogenic germs of these maladies will be discovered, as in the cases of splenic fever, and relapsing fever, and a few others in which the specific bacterium has been already identified.

There is, however, another class of chemical transformations, which differ very considerably from all to which we have hitherto alluded. They, however, resemble these fermentations in the fact that they occur independently of any apparent change in the agents that produce them. The agents that produce them are not living organisms, but chemical substances, the result of the activity of living cells. As instances of this class of chemical transformations, the following may be taken: the change of starch into sugar by the ptyalin of the saliva, the change of proteids into peptones by the pepsin of the gastric juice, the change of fibrinogen into fibrin, when blood is shed, &c. &c. These changes are also included under the term fermentation.[1]

Fermentations may therefore be divided into two classes: first, those brought about by the organised ferments (*torulæ*, *bacteria*, &c.), and, secondly, those brought about by the unorganised ferments (pepsin, diastase, &c.). Each of these classes may be again subdivided, according to the nature of the chemical change produced.

Previous to 1838, the action of yeast was regarded as a catalytic one (Berzelius); that is to say, the influence of its mere presence causes a separation of the constituents of sugar, just as platinum black causes peroxide of hydrogen to give up an atom of its oxygen. A modification of this theory was proposed by Liebig in 1848; he gave the organisms associated with the change a secondary position, holding that they produced substances of a chemical nature which were the true ferments; and he considered that the molecular vibrations of these ferments caused a rearrangement of the atoms of the substance

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[1] Sheridan Lea suggests the term zymolysis for this variety of fermentation (*Journ. of Physiol.* 1890, p. 254). Sir W. Roberts suggested the term enzymosis (*Proc. Roy. Soc.* vol. xxxi. p. 145) many years ago for the same processes.

CHAPTER XII

FERMENTATION

It has been known since very ancient times that sweet juices of fruits, more particularly of the grape, can be made to undergo certain changes, the result of which is, that the juice is no longer a sweet innocuous liquid, but possesses intoxicating properties. During the occurrence of this change the clear fluid becomes turbid, and its surface is covered by bubbles or froth. This latter phenomenon attracting special attention, the name fermentation[1] was given to the process. We now know that the change consists in the transformation of the sweet substance sugar into other materials, of which the most abundant are alcohol, the body possessing the intoxicating properties, and carbonic acid, the evolution of which causes the frothing. The turbidity of the liquid is caused by the presence of numerous unicellular organisms, (*torulæ*)[2] which increase rapidly by a process of budding.

This form of fermentation is now usually called the alcoholic fermentation, for it has been found that other chemical changes of the same nature may properly be included under the general term fermentation. As instances of these, the souring of milk, the transformation of urea into ammonium carbonate, &c., may be cited. The complex series of changes which are accompanied by the formation of malodorous gases, and which are known as putrefaction, come also into the same category. In all these instances the transformation is accompanied by the presence of unicellular organisms, which correspond to the *torulæ* of the alcoholic fermentation; for instance, in putrefying material, various kinds of bacteria, undergoing rapid growth and multiplication, will be discovered. It was for a long time a matter of doubt, whether these organic growths were the cause, or result, or an accidental concomitant, of the fermentative process. But it is now almost universally acknowledged that the organisms are the cause of the fermentation. It has been shown that the growth of such organisms is accompanied with the fermentation in question, that such fermentation occurs only when the organisms are growing, and stops when the organisms are removed or killed.

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[1] From *fervere*, to boil.

[2] A list of a large number of fungi (Saccharomyces cerevisiæ, S. ellipsoideus, Mucor, Mycoderma, &c.) which excite the alcoholic fermentation will be found in a paper by Reess, *Bot. Untersuch. ü. d. Alcoholgährungspilze*; *see* also Schunck, *J. prakt. Chem.* lxii. 222.

This vitalistic theory of fermentation becomes especially important to the physiologist and pathologist when applied to disease. The 'germ theory,' as it is termed, explains the infectious or zymotic diseases by considering that the change in the system is of the nature of fermentation, and like the other fermentations we have mentioned, produced by particular forms of bacterium; the transference of the bacteria or their spores from one person to another constituting infection. This theory has not been fully verified for every infectious disease by the discovery of a specific microbe; many able investigators, however, consider it likely that the pathogenic germs of these maladies will be discovered, as in the cases of splenic fever, and relapsing fever, and a few others in which the specific bacterium has been already identified.

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[1] Sheridan Lea suggests the term zymolysis for this variety of fermentation (*Journ. of Physiol.* 1890, p. 254). Sir W. Roberts suggested the term enzymosis (*Proc. Roy. Soc.* vol. xxxi. p. 145) many years ago for the same processes.

In certain cases this view of Liebig has been justified; soluble ferments have been separated from the organisms, and these have the same action when the organisms are absent as when they are present. Thus yeast cells, in addition to causing the alcoholic fermentation, produce also an inverting ferment, that is, a ferment which transforms cane sugar into glucose; this ferment can be readily separated from the organisms (Barth,[1] Donath,[2] Lea,[3] &c.). The alkaline fermentation of urine, in which urea is converted into ammonium carbonate, is brought about by an organism very similar to yeast, and to it the name *torula ureæ* has been given. Here, again, a soluble ferment with the same power has been separated from the cells (Musculus,[4] Lea). But in the greater number of cases, attempts to separate such soluble chemical ferments have been unsuccessful, and thus attention has been more concentrated on the biological side of the problem. In the case of the alcoholic fermentation, Helmholtz, Mitscherlich, and others, showed that if the yeast cells were prevented from passing into a fermentable liquid by the interposition of an organic membrane, fermentation did not ensue. That the organisms themselves are absolutely necessary, is also shown by experiments with the *bacillus anthracis*, the specific microbe of anthrax or splenic fever. A cultivation of the bacillus inoculated into an animal causes the death of that animal by splenic fever; but if the bacilli be first carefully filtered off from the cultivation fluid, the filtrate is innocuous.[5]

If, however, it be freely admitted that the organisms themselves are the cause of the fermentation, the question still remains, how do they act? Do they live on the fermentable matter, and then excrete what we call the products of fermentation? This view is not tenable, because of the immense volume of the substances in which they produce changes; Pasteur considers that of the sugar acted upon by yeast only one per cent. is taken up by the yeast itself. Another view, which is probably more correct, is that the organisms produce, very much as Liebig supposed, a soluble ferment, which acts on the fermentable matter. This view, which has received the powerful support of Hoppe-Seyler, is at once confronted with the difficulties already mentioned, the chief of which is the inability of various observers to separate such soluble ferments from the organisms. It is, however, always unsafe, when results of experiments on any subject are negative, to assume that our knowledge upon that subject is complete and final. The inability of observers in the past to perform an experiment may be from lack of means or of knowledge; and it is possible that the presence of soluble ferments in places where their existence has been hitherto denied, may be demonstrated in the future.

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[1] *Ber. d. deutsch. chem. Gesell.* 1878, p. 474.

[2] *Ibid.* 1875, p. 795.

[3] *Journ. of Physiol.* vi. 136.

[4] *Compt. rend.* lxxxii. 333. *Pflüger's Archiv*, xii. 214.

[5] In such experiments the culture fluid employed has been beef-tea or a similar infusion. More recent experiments [by Wooldridge, Hankin, and Martin have shown that if the bacilli be grown in a fluid rich in proteids, they produce a poison, a solution of which causes anthrax (*see* p. 168).

The separation of the inverting ferment from yeast, and of the urea-ferment from the *torula ureæ*, is a step which may be the first in a series of discoveries. Sheridan Lea in his experiments, indeed, pointed out a possible explanation of the negative results of previous investigators. Both the urea-ferment and the inverting ferment were obtained by precipitation of the cells with alcohol, and subsequent extraction of the alcoholic precipitate with water, but neither is present in the fluid surrounding the cells during the progress of the change which they produce. This is probably due to the fact that ferments, being non-diffusible, are unable to pass from the protoplasm of the torula, through its surrounding investment of cellulose.

It has already been surmised that ferments are of the nature of the living proteids (p. 146); like other proteids they are indiffusible; this readily accounts for the fact they are not discoverable outside the cell wall; and like all living things their properties during life are different from those after death; this readily accounts for the fact that, with a few exceptions, they are not discoverable inside the cell wall, after the cell has been killed by alcohol. The few exceptions are probably those which are more robust, and withstand the action of alcohol better.

If this hypothesis be admitted, and until it is replaced by a better it must be admitted, the difference between organised and unorganised ferment action is this: an organised ferment is one which does not leave the living cell during the progress of the fermentation; an unorganised ferment is one which is shed out from the cells, and then exerts its activity. Probably the chemical nature of the ferment is in the two cases the same, or nearly the same.

If it be admitted that the ferments are proteid in nature, or something closely akin to proteid, and it be also remembered how imperfect our knowledge of the proteids is, it may seem a task from which one would shrink, to attempt to explain any further how the ferments act. The ferment actions however consist very largely in the transference of water, or of oxygen; and we happily have in the simpler regions of chemistry, examples of action which seem to be analogous to what we call ferment action in the vaguer regions of organic chemistry.

The most striking of the phenomena of fermentation are these:—

(1) A small amount of the ferment produces a change in an overwhelmingly large quantity of material. This is even more puzzling in the case of the unorganised than in that of the organised ferments. A needle prick, if the point of that needle is infected with the *bacillus anthracis*, will cause the animal so inoculated to die of splenic fever. The inoculated bacilli have the power of rapid multiplication, and so rapidly poison the whole of the blood. A minute fragment of rennet will cause curdling throughout a huge volume of milk. There is here, however, no such power of self-multiplication.

(2) The ferment itself takes no apparent part in the change produced, but, after having produced its action, can be used again to produce the same action in another mass of material.

The vibration theory of Liebig is only to a certain extent an explanation of these phenomena;[1] the changes taking place among the atoms composing the molecules of the ferment produce vibrations, which, acting on the molecules of the substance with which the ferment comes in contact, set up there similar molecular vibrations and rearrangements.

This is quite comparable to what is taking place around us every day in a social capacity. An irritable quick-tempered individual enters a room filled with pleasant people. The influence of his presence soon causes the whole assembly to become changed, and bad temper to rule supreme. The analogy to the case of a ferment is completed by the fact that the author of the change is himself unaltered, and is capable of producing the same action on another mass of material.

This homely comparison however helps us very little; it leads us into the regions of psychology, where the problems are even more complicated than in physiology. It will be of greater help to find comparisons in simpler chemical reactions which are well understood.

Take the case of the ordinary way in which oxygen is made. If one heats potassium chlorate ($KClO_3$), the oxygen comes off, and potassium chloride (KCl) is left behind. If, however, a little manganese dioxide be mixed with the chlorate in the first instance, the oxygen comes off much more easily, but the manganese dioxide is unaltered at the end of the experiment, and this is quite comparable to what occurs in the case of a ferment.

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[1] A 'contact theory' more recently advanced (*Watts' Dictionary*, 1889, vol. ii. p. 540) is that the enzymes raise the molecular temperatures of the decomposing molecules to the point at which their molecular equilibrium is destroyed; their decomposition is produced by rearrangement of energy, not by any increase or decrease of the amount present in the system.

Take another example; in the manufacture of ordinary oil of vitriol, sulphurous acid, atmospheric air, and steam are brought into contact with one another in a large leaden chamber. These three substances alone would suffice to form sulphuric acid ($SO_2 + O + H_2O = H_2SO_4$), but the action would be a slow one. The combination is hastened by the presence of a small quantity of nitrous acid (N_2O_3). The sulphurous acid (H_2SO_3) combines with the nitrous acid, and is then decomposed into sulphuric acid (H_2SO_4) and nitric oxide (N_2O_2).

$$\underset{\text{[sulphurous acid]}}{H_2SO_3} + \underset{\text{[nitrous acid]}}{N_2O_3} = \underset{\text{[sulphuric acid]}}{H_2SO_4} + \underset{\text{[nitric oxide]}}{N_2O_2}$$

The nitric oxide left combines instantly with oxygen, to form nitrous acid again, which in turn undergoes the same decomposition with sulphurous acid. Thus the nitric oxide serves as an oxygen carrier, and as it is continually being recovered, and itself taking no part in the composition of the final product (sulphuric acid), a small quantity will last an indefinite time, and always be ready to perform the same office. Here again it plays the part of a ferment.

Take another example, this time from organic chemistry; namely the action of sulphuric acid in the manufacture of ether from alcohol. If one distils together alcohol and sulphuric acid, ether and water will be found in the distillate, and the sulphuric acid apparently unchanged in the retort; and the same quantity of sulphuric acid can be used over and over again, to break up an indefinite quantity of alcohol. Now if the action of the sulphuric acid had not been understood, as it was not until comparatively recent times, the reaction would have been still looked upon as puzzling, and described as catalytic. We do, however, understand how sulphuric acid acts.

The first reaction that takes place may be denoted in this way. We start with alcohol and sulphuric acid:—

$$\begin{array}{ll} HHSO_4 & \text{sulphuric acid,} \\ \hline OH(C_2H_5) & \text{alcohol.} \end{array}$$

When these come together, the vertical line represents the products of their interaction; they split into

$$\begin{array}{r|l} H & HSO_4 \\ OH & (C_2H_5) \\ \text{water} & \text{ethyl-hydrogen sulphate} \end{array}$$

water, which comes over in the distillate, and ethyl-hydrogen sulphate.

The ethyl-hydrogen sulphate reacts with more alcohol, and, the vertical line again indicating the way in which the atoms are rearranged, we have

$$\begin{array}{c|l} (C_2H_5) & HSO_4 \\ C_2H_5O & H \\ \text{ether} & \text{sulphuric acid} \end{array}$$

sulphuric acid, again ready to be split up as before, and ether, which distils over. In this reaction the sulphuric acid acts as probably ferments do in fermentation. Apparently they are unchanged at the end of the reaction; probably they have acted in some such way as the nitric oxide or the sulphuric acid do in the examples just given. Probably they play the part of an oxygen carrier, or a water carrier, and then in the later stages of the reaction are deprived of their extra oxygen or water, and thus appear the same as before the reaction began.

Lastly, an example may be taken from physiology itself; the example is that of the action of hæmoglobin; it comes to the lungs in the venous blood, is converted there into oxyhæmoglobin, takes the oxygen to the tissues, and returns as it started, in the condition to act over and over again as an oxygen carrier.

This action of hæmoglobin is not generally called a ferment action, but it appears to me to be clearly in the same category of phenomena. We do not call it a ferment action, because we understand it; when we attain to a similar accurate understanding of the action of pepsin, and of bacteria, we shall probably cease to call them ferment actions, and reserve that term for what we do not understand as a convenient cloak for ignorance. The action of sulphuric acid in etherification is no longer cloaked under the similar term *catalysis*. There seems no reason why in the future we may not attain to as accurate knowledge concerning ferment actions as chemists have arrived at in connection with many formerly so-called catalytic phenomena.

We have thus a series of occurrences in chemistry, starting with the simple catalytic processes of inorganic chemistry, and ending with the ferment processes of physiological chemistry, probably differing only in the complexity of the substances taking part in them. Ferment activity is a manifestation of protoplasm in a living condition; and I regard it as possible that, by working out ferment actions in the light of the simpler catalytic actions, we shall obtain an insight into the explanation of other still more complex vital actions.

A step to the better knowledge of fermenting processes has been made by Hoppe-Seyler,[1] who has pointed out that the oxidation in which the action often apparently consists is not a direct one, but rather of the nature of reduction.

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[1] The most recent exposition of Hoppe-Seyler's views in this direction will be found in the *Zeit. physiol. Chem.* x. 36.

Thus in the lactic and alcoholic fermentation, and in putrefaction, there is a liberation of hydrogen, and this nascent hydrogen combines with an atom of oxygen from ordinary oxygen (O_2) to form water ($H_2 + O_2 = H_2O + O$). The nascent oxygen (O) thus liberated, oxidises any oxidisable substance present, or it may unite with hydrogen to form water, or oxygen to form ozone (O_3). But if on the other hand the nascent hydrogen meets with no free oxygen, it takes the oxygen from organic substances, that is, reduces them. Thus in putrefying liquids, oxidation may be proceeding in the upper portions where there is free access of atmospheric oxygen, and reduction in the lower layers where free oxygen is absent.

It is probable that some of the changes occurring during the nutrition of living cells are similar to these fermentations. The nascent hydrogen liberates nascent oxygen, which then oxidises oxidisable material. The following hypothetical formula would represent what occurs; supposing n is oxidisable material, then

$$HH + O_2 + n = H_2O + On.$$

THE UNORGANISED FERMENTS

These substances can be extracted from the cells in which they occur by water, dilute acids or alkalis, salt solution, or glycerine. They are precipitated from such extracts, or from the secretions in which they occur, by alcohol, or by saturation with ammonium sulphate,[1] or by lead acetate. The precipitate so obtained is proteid in nature,[2] or closely allied to proteid. On drying this precipitate a colourless, tasteless, amorphous powder is obtained.

These ferments may be arranged, according to their action, into the following classes:—

1. Proteolytic: those which change proteids into peptones. This is probably a process of hydration, as it can be also brought about by other hydrating agencies, such as boiling with dilute mineral acids, or superheated steam.

Examples: pepsin, trypsin, papain.

2. Amylolytic: those which change amyloses (starch, glycogen) into sugars. This also is a hydration.

Examples: ptyalin, amylopsin, diastase.

3. Steatolytic: those which split fats into fatty acids and glycerine.

Examples: ferments in pancreatic juice and bile.

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[1] Krawkoff, *J. Russ. Chem. Soc.* 1887, p. 387.

[2] Elementary analyses have been made of various ferments by Schmidt, Schlossberger, Hüfner, and others. Much the same results have been thereby obtained as in the case of proteids.

4. Inversive: those which convert cane sugar into glucose.

Examples: invertin of intestinal juice, and of yeast cells.

5. Emulsin[1] or synaptase: a ferment which converts glucosides (amygdalin, salicin, &c.) into glucose, and other compounds. Myrosin is a very similar ferment.

6. Coagulative. Examples: fibrin ferment, myosin ferment, rennet, ferment from *Withania coagulans* which acts like rennet (Lea). A rennet-like ferment is obtained from certain other plants,[2] and certain bacteria.[3]

There are other fermentations, such as the conversion of glucose into mannite, or of glycerine and mannite into alcohol by the action of putrefying nitrogenous organic matter, which have been described, but which are of little importance to the physiologist.

The preceding classification is found to be very useful from a physiological standpoint. In many instances the same chemical change, which in all cases appears to be of the nature of hydrolysis, may be effected by the action of ordinary chemical reagents, such as dilute mineral acids, or caustic alkalis. Hoppe-Seyler[4] has accordingly classified ferments from a chemical standpoint as follows:—

a. ***Ferments which act like dilute mineral acids at*** 100° *C.*:—

i. Change of starch or glycogen into dextrin and grape sugar.[5]

$$\underset{\text{[starch]}}{C_{24}H_{40}O_{20}} + 3H_2O = \underset{\text{[dextrin]}}{C_6H_{10}O_5} + \underset{\text{[glucose]}}{3C_6H_{12}O_6}$$

ii. Change of cane sugar into dextrose and levulose (inversion).

$$\underset{\text{[cane sugar]}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{[dextrose]}}{C_6H_{12}O_6} + \underset{\text{[levulose]}}{C_6H_{12}O_6}$$

iii. Change of various benzol-glucosides into sugar, and simpler benzol-derivatives by the action of emulsin (*see* p. 109).

$$\text{Example: } \underset{\text{[salicin]}}{C_{13}H_{18}O_7} + H_2O = \underset{\text{[sugar]}}{C_6H_{12}O_6} + \underset{\text{[saligenin]}}{C_6H_4\left\{\begin{matrix}CH_2OH\\ OH\end{matrix}\right.}$$

iv. Decomposition of sulphur-containing glucosides into sugar, sulphuric acid, and oil of mustard, by the action of myrosin.[6]

$$\text{Example: } \underset{\substack{\text{[potassium}\\ \text{myronate]}}}{C_{10}H_{18}NS_2O_{10}K} = \underset{\text{[sugar]}}{C_6H_{12}O_6} + \underset{\substack{\text{[hydrogen,}\\ \text{potassium}\\ \text{sulphate]}}}{HKSO_4} + \underset{\substack{\text{[oil of}\\ \text{mustard]}}}{C_4H_5NS}$$

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[1] Emulsin was prepared in a very pure condition by Aug. Schmidt (*Inaug. Diss. Tübingen*, 1871). He found that it had the following percentage composition: C, 48·76; H, 7·13; N, 14·16; S, 1·25; O, 28·70.

[2] E.g. artichokes, black pepper, &c. *See Watts' Dictionary*, vol. ii. (1889), p. 545.

[3] Warington, *Journ. of Chem. Soc.* 1888, p. 737.

[4] *Physiol. Chem.* (1881), p. 116.

[5] The above formula is Hoppe-Seyler's after Musculus. Brown and Morris give a different equation (*see* p. 104).

[6] In the above equation the process is apparently not one of hydrolysis, but it seems

b. *Ferments which act like caustic alkalis at a higher temperature. Fermentative saponification:—*

i. Decomposition of ethers (fats) into an alcohol (glycerine) and an acid (fatty acid).

$$\text{Example: } \underset{\text{[tri-olein]}}{C_{57}H_{104}O_6} + 3H_2O = 3(\underset{\text{[oleic acid]}}{C_{18}H_{34}O_2}) + \underset{\text{[glycerine]}}{C_3H_8O_3}$$

ii. Decomposition of amido-compounds with absorption of water. Examples:

$$(1)\ \underset{\text{[urea]}}{CON_2H_4} + H_2O = \underset{\text{[ammonium carbonate]}}{(NH_4)_2CO_3}$$

$$(2)\ \underset{\text{[hippuric acid]}}{C_9H_9NO_3} + H_2O = \underset{\text{[glycocine]}}{C_2H_5NO_2} + \underset{\text{[benzoic acid]}}{C_7H_6O_2}$$

$$(3)\ \underset{\text{[taurocholic acid]}}{C_{26}H_{45}NSO_7} + H_2O = \underset{\text{[taurine]}}{C_2H_7NSO_3} + \underset{\text{[cholalic acid]}}{C_{24}H_{40}O_5}$$

(4) The decomposition of proteids and albuminoids (gelatin, chondrin, &c.) into leucine, tyrosine, &c., brought about by the pancreatic ferment—trypsin.

It will be seen, on glancing through these enumerations of the unorganised ferments, that the greater number of those occurring in animals are found in the alimentary canal, and are concerned in the digestion of food. They all act best at a little over the temperature of the body (40° C.), their activity is hindered by a lower temperature, and they are destroyed by a high temperature. In a dry state pepsin and trypsin may be heated to 170° without harm,[1] but in a moist state a temperature below 100° C.[2] is sufficient to destroy them.

All fermentative processes require the presence of a certain amount of water. Free acid is harmful, except in a few cases, e.g. gastric digestion. The caustic alkalis also when present in more than very minute proportions hinder fermentation; as also do salts of the heavy metals, and ether, and chloroform in some cases.

Quite small percentages of neutral salts (such as 0·004 per cent. of the sulphate of sodium, potassium, ammonium, or magnesium, 0·02 of various urates, 0·01 of sodium chloride or phosphate) have a very considerable inhibitory effect when pure solutions of pepsin or trypsin are used (Nasse,[3] Heidenhain,[4] A. Schmidt,[5] E. Stadelmann[6]).

Bert and Regnard state that organised ferments are killed by

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probable on further investigation that the formula of myronic acid may be modified, and that the ferment change will be found to be also one of hydrolysis (Will and Körner, *Liebig's Annalen*, cxxv. 263). By hydrolysis, one means the fixation of the elements of water, followed by decomposition into simpler products. The term should be distinguished from hydration, in which there is no such subsequent decomposition.

[1] Huppe, *Chem. Centralblatt*, 1881, p. 745.

[2] The critical temperature at which the soluble ferments are destroyed varies with the different enzymes; the range of temperature is approximately 50°–75° C.

[3] *Pflüger's Arch.* xi. [4] *Ibid.* x. [5] *Ibid.* xiii. [6] *Zeit. Biol.* xxv. 208.

peroxide of hydrogen, and unorganised ferments not. Schützenberger and Dumas state that borax destroys the activity of the unorganised, but not that of the organised, ferments.

THE ORGANISED FERMENTS

Gay-Lussac showed that boiled grape-juice introduced into the Torricellian vacuum of a barometer remained free from fermentative change for an indefinite time, but that on the admission of a bubble of air, fermentation soon commenced. Schwann (1838) showed that, if the bubble were admitted to the vacuum through a red-hot tube, fermentation did not occur. This experiment demonstrated clearly that, whatever it was in the air that caused fermentation, it was destroyed by heat. In the same year Schwann pointed out the vegetable nature of the yeast cells which had been seen by Leeuwenhoek so long ago as 1680; Schwann showed too that they grew in saccharine solutions, and for the first time it was asserted that fermentation depended on the action of living things. In the seventeenth century Stahl had remarked that fermentation and putrefaction were essentially the same; this, however, was not verified until Schwann demonstrated (1) that if air were excluded from boiled putrescible fluids, they did not putrefy; (2) that if air were subsequently admitted, putrefaction soon set in; (3) that if the air had been previously passed through red-hot tubes, no putrefaction occurred; (4) that the putrefying fluids always contained bacterial growths; (5) that certain substances which we now call antiseptics, such as corrosive sublimate, which destroy organic life, put a stop to putrefaction, presumably by destroying the bacteria.

There were many other observations made, all tending to the same end, namely, that it was not air, but something in the air, that caused both fermentation and putrefaction; among these may be mentioned that of Hofmann, who showed that cotton wool will filter off from the air the material in question; and that of Mitscherlich, who showed that yeast loses its power after thorough filtration and removal of the yeast cells.

In 1857 Pasteur showed that each particular kind of fermentation was connected with the growth and development of a special organism; one organism producing the alcoholic, another the lactic, another the acetous fermentation, and so on. By introducing the method of cultivating organisms in certain special fluids particularly adapted to the wants of one kind, and only one kind, of organism, he was able to separate different organisms from one another, and thus to investigate their individual properties, by inoculating them into previously sterilised putrescible or fermentable fluids, or into the body of certain animals.

This method has in the hands of other investigators (Koch, Klein, &c.) been much elaborated, and bacteriology is now a science in itself. The method of pure cultivations has attained to a great pitch of perfection, and by it organisms which are apparently the same to the microscope can be divided into different kinds, according to their manner of growth and their physiological effects. The value of the method has been of most value in connection with the microbes of disease.

The general principles of the method may be briefly summarised here, but for full particulars the reader is referred to any of the excellent works on the subject now published.

The medium in which bacteria are to be grown may be either solid or liquid. Good liquid media are sterilised milk, serum or hydrocele fluid, or a bouillon made by boiling 500 grammes of beef in a litre of water for 45 minutes; this is rendered alkaline by carbonate of soda, filtered, and sterilised by exposure to the temperature of 100° C. for about an hour on three successive days.[1] Some culture liquids are simpler; thus *Pasteur's fluid* is 1 part ammonium tartrate, 10 parts cane sugar, and the ash of 1 part of yeast in 100 parts of water. *Cohn's fluid* is 0·5 grm. potassium phosphate, 0·5 grm. magnesium sulphate, 0·05 calcium phosphate, and 1 grm. ammonium tartrate in 100 grms. of water.

Among solid media, which are more suitable for pure cultivations, the following may be mentioned:—

a. The cut surface of a boiled potato or boiled white of egg sterilised by being washed with a solution of corrosive sublimate.

b. Meat juice, to a litre of which is added 10 grms. of commercial peptone, 5 grms. of sodium chloride, and 100 grms. of pure gelatin. The mixture is heated, made slightly alkaline with sodium carbonate, filtered hot into test-tubes sterilised by discontinuous heating, and the tubes plugged with sterilised cotton wool.

c. Instead of gelatin in the above, agar-agar (prepared from seaweed) may be used. This gelatinises at a higher temperature than gelatine, and so is suitable for the cultivation of organisms which grow only at temperatures approaching that of the body.

d. Blood serum made firm by heating to 68° C. for an hour. This is sterilised by raising it to 56° C. for two hours daily for eight days.

All instruments used in experimentation must be sterilised—metallic instruments by the Bunsen flame, glass instruments by placing them in an oven at 160° C.

To inoculate a new tube or flask with a definite organism that has been growing previously in a culture tube: push the point of a freshly drawn out capillary pipette through the cotton wool plug until it reaches the culture fluid or solid; a small drop ascends the tube of the pipette. Withdraw the pipette and similarly push it into the material at the bottom of the second tube—that which is to be inoculated. The pipette is withdrawn, and the tube placed in an incubator at the temperature at which the micro-organism grows best.

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[1] This 'discontinuous sterilisation,' as it is called, was introduced by Tyndall (*Floating Matter in the Air*, 1881), who found it more efficacious than prolonged heating. The reason is that the spores resist heat much more powerfully than the fully grown bacteria; the spores not killed by the first heating germinate before the second boiling when they are killed, while some which may not have germinated will have done so by the time of the third boiling.

The separation of organisms may be effected by one of three methods:—

1. Kleb's method of fractional cultivation.
2. Lister's and Nägeli's dilution method.
3. Koch's method of plate cultivation.

1. Fractional cultivation.—If a trace of culture fluid containing several organisms be inoculated into a series of new tubes containing different nourishing materials, it will be found after 24 to 48 hours that probably one species in each tube—i.e. the one that grows best in that particular medium and at that particular temperature—will have increased enormously, and that the others have made little or no progress. Inoculate a new culture tube with a trace of this cultivation; the chances are that you inoculate one kind of organism only, viz. the most abundant; but, to be certain, the process should, after 24 hours, be repeated, and if necessary repeated again. The naked eye appearances of the cultivation, coloration, or liquefaction of the medium, the formation of a pellicle, microscopic appearance, and many other conditions, soon indicate when a desired single species is obtained.

2. The method of dilution.—The original culture fluid containing several species is greatly diluted with sterile salt solution, or some other indifferent fluid: with droplets of this new tubes are inoculated. It is probable that, owing to the great dilution, the droplet used contains only one organism. This chance is increased by repeating the process several times in succession. This method may be very successfully combined with that of fractional culture.

3. Plate cultivation.—A test-tube of sterile nutritive gelatine is liquefied by gentle heat, and inoculated with a trace of the bacterial mixture, either by a capillary pipette or by the point of a previously over-heated and cooled platinum wire; this is well mixed and expeditiously poured into a sterilised shallow glass dish, and covered with another glass dish; both are then placed under a bell-jar, the interior of which is kept moist by a piece of wet blotting-paper, and the whole incubated at a suitable temperature. In a few days each species of bacterium will start a separate colony, differing in shape, colour, size, and general appearance, from the others. By reinoculation of gelatine tubes from these, pure subcultures of the different species can be obtained.

Modifications of these various methods are used for the inoculation of nutrient material with blood, juices, and tissues of animals, and in the examination of water and air for micro-organisms.[1]

Another large branch of the science of bacteriology is that relating to the microscopic preparation and methods of staining of micro-organisms, which has now reached a great degree of elaboration.

Micro-Organisms may be classified in many different ways.

They may be classified morphologically; they all belong to one of the lowest groups of fungi called the *Schizomycetes*. They are devoid of chlorophyll, and multiply usually by fission, but in some (many bacilli) by a process of spore formation. The yeast cells, which are considerably larger than most other globular forms, multiply usually by budding. The forms assumed by bacterial growths are :—

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[1] For full particulars see *Klein's Micro-Organisms and Disease.*

(*a*) Globular; termed micrococcus.

(*b*) Rod-like; or bacillus.

(*c*) Filamentous; either single filaments, or composed of bacteria remaining attached after division (Leptothrix).

(*d*) Spiral; termed vibrio, or if the sinuosity is very great, spirillum.

(*e*) Plates or tablets formed by the irregular branching of cells, the branches remaining attached (*sarcina*).

As a rule a microbe retains the same form generation after generation; but occasionally, as in cladothrix, a micrococcus form may become rod-like or filamentous at another stage (pleomorphism). Dense swarms sometimes occur in which the bacteria become fixed in a matrix of their own swollen, contiguous cell walls, and pass into a resting state as a so-called *zooglœa*.

Another classification of these growths may be made according to whether the organisms are aërobic, or anaërobic. In 1864 Pasteur observed that the butyric acid ferment can live and multiply in a saline fluid containing sugar and calcium lactate in the absence of free oxygen (anaërobic); on the other hand, other growths like the bacterium aceti require oxygen (aërobic). In a mixture of bacteria Engelmann showed that some species gather close to a bubble of air, others come near it when it has lost some of its oxygen, and others keep away from it altogether. Most fermentative organisms are capable however of assuming two conditions : one aërobic, the other anaërobic ; it is in most cases in the latter condition that an organism carries on the work of fermentation, as it has to remove oxygen from the fermentable material.

A third classification may be made on the basis of the effects caused by the growths of the micro-organisms :—

(*a*) Those associated with known chemical processes ; such as the yeast plant, the bacterium lactis, the bacterium aceti, the micrococcus ureæ, &c.

(*b*) Those associated with the putrefaction of organic matter ; these are various forms of micrococcus, bacterium termo, bacterium subtile (the Hay bacillus), &c.

(*c*) Those chiefly remarkable for producing colour ; such as *bacterium rubescens*, the peach-coloured bacterium, *B. syncyanum* of blue milk, *B. æruginosum* of green pus, the *micrococcus prodigiosus* of red bread, and many others.

(*d*) Those which produce disease when grown within the living body ; among these may be mentioned the bacillus anthracis of splenic fever, the spirillum of relapsing fever, bacilli in various forms of septicæmia, in fowls' cholera, the comma bacillus of Asiatic cholera, and many suspected growths in various zymotic diseases, as smallpox, diphtheria, scarlet fever, typhoid, leprosy, rabies, &c. (*See* also Chapter XVI, Blood in Disease.)

As is seen from the preceding list, a chemical classification is at present impossible ; in a few cases, as in the alcoholic fermentation, the lactic fermentation, or the acetic fermentation, &c., the decomposition can be represented by means of a chemical equation ; in other cases the substances produced may be identified, but the chemical decompositions by which they are brought into existence are unknown; this is particularly the case when we have to deal with proteids. In other cases still, particularly in disease germs, even the products of fermentation are still unknown. The recent discovery of the importance of animal alkaloids (ptomaines and leucomaines) has led in certain cases to the discovery that it is these substances which are the chemical poisons so long sought after. In other cases the poisons are proteid in nature.

The following classification of the action of organised ferments according to their chemical action, is a completion of Hoppe-Seyler's arrangement, part of which has already been given in connection with the unorganised ferments (p. 159).

I. *Ferments which change anhydrides into hydrates or cause hydrolysis* :—

a. Ferments acting like dilute mineral acids at 100°. These appear to be all unorganised.

b. Ferments acting like caustic alkalis at a higher temperature.

i. Decomposition of fats into glycerine and fatty acids. This appears to occur not only with the unorganised pancreatic ferment, but also during putrefaction, presumably by the action of bacteria.

ii. Decomposition of amido-compounds with absorption of water.

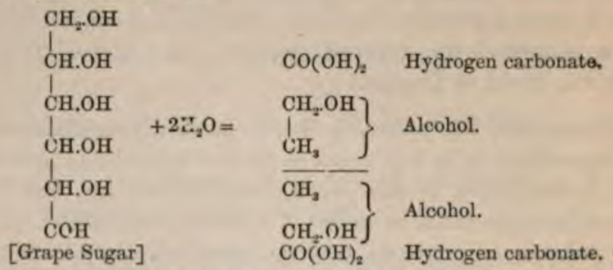
The four examples already given (p. 160) are brought about also by putrefactive organisms ; the decomposition of urea by the m. ureæ, through the intermediation of a soluble ferment ; the decomposition of hippuric acid, taurocholic acid (and glycocholic acid more slowly) by putrefactive bacteria ; and the decomposition of proteids and albuminoids into leucine, tyrosine, &c., is brought about, not only by the unorganised ferment trypsin, but also by putrefactive bacteria, such as occur, for instance, in the intestinal canal.

II. *Fermentations in which there is transference of oxygen from the hydrogen to the carbon atoms.*

a. The lactic acid fermentation. The decomposition of milk-sugar, inosite, and other carbohydrates, into lactic acid. The ferment is associated with the presence of a micro-organism—the *bacterium lactis*. But other fungi—e.g. the spores of *penicillium*—will bring about the same result (*see* also Milk).

b. The alcoholic fermentation.[1]

The transference of the oxygen from the hydrogen to the carbon atoms is thus depicted by Hoppe-Seyler:—



$$\begin{array}{lcll} CH_2.OH & & & \\ | & & & \\ CH.OH & & CO(OH)_2 & \text{Hydrogen carbonate.} \\ | & & & \\ CH.OH & & \left.\begin{array}{l} CH_2.OH \\ | \\ CH_3 \end{array}\right\} & \text{Alcohol.} \\ | & +2H_2O= & & \\ CH.OH & & & \\ | & & & \\ CH.OH & & \left.\begin{array}{l} CH_3 \\ | \\ CH_2.OH \end{array}\right\} & \text{Alcohol.} \\ | & & & \\ COH & & & \\ \text{[Grape Sugar]} & & CO(OH)_2 & \text{Hydrogen carbonate.} \end{array}$$

c. Many cases of putrefaction come under this head[2]:—

i. Of simple inorganic compounds:

Examples: (1) $\underset{\text{[calcium formate]}}{(CHO_2)_2Ca} + H_2O = \underset{\text{[calcium carbonate]}}{CaCO_3} + CO_2 + 2H_2$

(2) $\underset{\text{[calc, acetate]}}{(C_2H_3O_2)_2Ca} + H_2O = CaCO_3 + CO_2 + \underset{\text{[marsh gas]}}{2CH_4}$

ii. Of organic compounds:—

(1) $\underset{\text{[calc. lactate]}}{(C_3H_5O_3)_4Ca_2} + H_2O = CaCO_3 + 3CO_2 + 4H_2 + \underset{\text{[calc, butyrate]}}{(C_4H_7O_2)_2Ca}$

(2) $\underset{\text{[cellulose]}}{nC_6H_{10}O_5} + nH_2O = \underset{\text{[carbonic acid]}}{3nCO_2} + \underset{\text{[marsh gas]}}{3nCH_4}$

(3) Calcium malate yields carbonate and lactate of lime.

(4) Glycerine with calcium carbonate and putrefying fibrin yields, on exclusion of oxygen, no alcohol, but butyric, butyro-acetic, and succinic acids; probably lactic acid is first formed, which is then decomposed into carbonic and butyric acids; the butyro-acetic acid arises from reduction; the formation of succinic acid is difficult to understand.

(5) Proteids are easily decomposed by putrefactive organisms. Insoluble proteids, like fibrin, are first dissolved, forming a solution of globulin. Solutions of proteid appear to undergo first a change like that produced by digestion with the formation of albumoses and peptone. Then amido-acids (leucine, tyrosine, &c.), fatty acids, ammonia, carbonic acid, amines, and in certain cases indole and skatole, are formed.

Another classification of ferments has been made according as they produce acid bodies (acetic acid from alcohol, lactic acid from sugar, &c.), or basic products (urea from ammonia, ptomaines from proteids). Such classifications are necessarily incomplete, but Hoppe-Seyler's is especially useful as showing what the basis of a scientific chemical arrangement should be. In addition to these forms of fermentation the list may be completed by the mention of:—

1. The acetous fermentation; the conversion of alcohol into acetic acid

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[1] Other alcohols (propyl, butyl, &c.) may be produced under suitable conditions.

[2] These cases have been examined by Hoppe-Seyler by mixing the substance in question with small quantities of sewer mud; the gases that come off are collected and analysed.

($C_2H_6O + O_2 = C_2H_4O_2 + H_2O$), brought about by the mycoderma aceti and the bacterium aceti.

2. Nitrifying organisms which occurring in the soil convert ammonia into nitric and nitrous acids, and so lead to the formation of nitre in nature (Warington).[1]

3. Sulphur bacteria (Winogradsky),[2] i.e. bacteria which in the presence of free hydrogen sulphide oxidise sulphur, forming sulphuric acid. Some of these bacteria are colourless; others produce pigments such as the B. rubescens (Lankester), which produces the chromophyll called bacterio-purpurin. This bacterium is called B. photometricum by Engelmann, on account of its behaviour in different parts of the spectrum.

4. Bacteria which produce ptomaines. The origin of an alkaloid (neurine) from lecithin is easily understood, since the constitution of lecithin is known. Lecithin, which is of very widespread occurrence in the body, is a compound of neurine (a poisonous alkaloid) with distearyl-glycero-phosphoric acid. There is however no doubt that bacteria may produce alkaloids from proteids. A theoretical explanation of how this may occur has been given by Latham,[3] on the supposition that his theory of the constitution of albumin (*see* p. 116) is correct.

The growth and development, and the associated fermentive activity, of organised ferments are influenced by various physical and chemical reagents.

Temperature.—They grow best at 35°-40° ; but different organisms vary considerably with regard to their optimum temperature. Boiling kills most bacterial growths ; the spores of bacilli are said however to have withstood a temperature of even 110°C. A temperature a good deal below boiling, 60°-70°, kills most microbes. On the other hand a low temperature −78°C. (Melsens[4]), −70° for 20 hours (Pictet and Young[5]), −83° for 100 hours (McKendrick and Coleman[6]) stops the activity of the organisms, but does not kill them ; for after thawing they resume work again.

Water.—A certain amount of moisture is necessary for their activity ; but spores may be dried, and will remain dormant for months and years, resuming activity when moistened.

Light.—Certain spores are killed by brilliant sunshine. Other forms are more active in certain regions of the spectrum than in others (Engelmann[7]).

Chemical reagents.—This leads us to allude to what is now a large and important branch of commercial industry and medical and surgical practice, known as antisepsis. The most powerful antiseptics, those that kill the bacteria and so prevent putrefaction and fermentation, are corrosive sublimate, carbolic acid, quinine, chlorine, and mineral acids. In the preservation of food-stuffs, sugar, salt, spirit, vinegar, borax, &c., a frequently used ; and cold chambers are now very generally employe

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[1] *Journ. Chem. Soc.* vols. xxxiii. xxxv. xlv. and li. Certain organisms appear to have the opposite effect, the reduction of nitrates to form ammonia (Frankland, *Ibid.* liii. p. 373).

[2] *Botan. Zeitung*, 1887, No. 31–37.

[4] *Compt. rend.* lxx. 629.

[3] *Proc. Roy. Inst. Gt. Britain*, 1885.

[5] *Lancet*, 1888, vol. ii. p. 751.

[6] *Ibid.* xcviii. 747.

[7] *Pflüger's Archiv*, xxx. 95 ; xlii. 183.

A remarkable fact concerning the ferments is, that the substanc they produce in time put a stop to their activity ; thus the alcoh produced, by yeast, the phenol, cresol, &c., produced by putrefacti organisms, are themselves antiseptics, which ultimately kill th organisms that produce them.

THE CHEMICAL POISONS PRODUCED BY BACTERIA

There has been no micro-organism that has received so much attention as the bacillus anthracis, but it is only within the last few months that we have obtained any accurate knowledge of the poisons which it produces. Wooldridge[1] was the first to show that if the bacilli are grown in a culture fluid containing proteid (he used an alkaline solution of tissue-fibrinogen), a substance is produced which, if inoculated into animals, renders them immune to anthrax. Hankin[2] then demonstrated that a small dose of an albumose produced by the activity of the bacilli is capable of protecting animals from the disease. Dr. S. Martin[3] hit upon the happy idea of growing the bacilli in solutions of pure alkali-albumin. Failure to find a chemical poison on the part of most previous investigators has doubtless arisen from the fact that they have employed infusions like beef-tea, which contain hardly any proteid, and so are very different from the blood, in which the growth of the microbe leads to the death of the animal. After filtering off the bacilli the filtrate was found to contain leucine and tyrosine, an alkaloid and three proteids, proto- and deutero-albumose, and a trace of peptone. The alkaloid forms salts, which were prepared in a crystalline form. The albumoses are strongly alkaline; they are not so toxic as the alkaloid, and Martin suggests that the alkaloid is in a nascent condition in the albumose molecule. The symptoms produced by the alkaloid are like those produced by the bacillus; after death, however, the organs of the animal are quite free from bacilli. From these researches we can conclude that the bacillus produces two substances, one of which is actively poisonous, and another which in small doses is protective. To use terms which are now being extensively employed, this microbe produces a *toxine* or poison, and a *vaccine* or protective principle. Such a supposition underlies the practice of vaccination and other forms of protective inoculation. It does not, however, appear to be a necessary part of this hypothesis that the toxine and the vaccine should be distinct substances. In certain cases, it may be that small doses of the former act as the latter.

The production of poisonous proteids by bacterial activity has also received attention in Germany. Thus Brieger and Fränkel[4] have investigated principally Löffler's bacillus of diphtheria, and have obtained a proteid which, when injected into rabbits, produces diphtheritic symptoms. This and similar proteids obtained from other bacterial growths they designate *toxalbumins*. They, however, appear to be albumoses rather than albumins. Brieger and Fränkel have overlooked the possibility that these poisonous proteids may contain an alkaloid closely bound to them, or in a nascent condition within their molecules.

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[1] *Proc. Roy. Soc.* xlii. 312; *Arch. f. Anat. u. Physiol., physiol. Abth.* 1888, 527.

[2] *Brit. Med. J.* Oct. 12, 1889. Hankin has also found that other proteids (especially cell globulin) have the power of killing certain pathogenic micro-organisms (*Proc. Roy. Soc.* May 22, 1890).

[3] *Proc. Roy. Soc.* May 22, 1890.

[4] *Berlin. klin. Wochenschrift*, April 1890.

CHAPTER XIII

PTOMAINES AND LEUCOMAINES

The word *ptomaine* (πτῶμα=corpse) was originally employed to designate those products of putrefaction which give the reactions of vegetable alkaloids, and have more or less of a poisonous action. It was subsequently found that similar alkaloids are formed during the life of animal organisms; these are termed leucomaines (λεύκωμα=white of egg; the word signifies that they are derived from proteids).

The term alkaloid was at one time applied to any organic base. It is now usually restricted to organic bases which are of vegetable origin, and produce marked toxicological effects; thus, such substances as ethylamine, asparagine, leucine, and other amido-acids are not classed as alkaloids, though in many of their properties they are basic. All the alkaloids contain nitrogen, and all, except coniine, nicotine, and sparteïne, contain oxygen. These three alkaloids are volatile, the others are fixed. The commonest and best known of the fixed alkaloids are aconitine from aconite, atropine from belladonna, strychnine and brucine from nux vomica, quinine and cinchonine from cinchona bark, morphine and codeine from opium, theine from tea and coffee, theobromine from cocoa.

Chemically, these substances are compound ammonias—that is, ammonias in which one, two, or three equivalents of hydrogen are replaced by radicles. They are thus analogous to the amines.

Methylamine is ammonia in which one H is replaced by methyl; ethylamine by ethyl.

$$\underset{\text{[ammonia]}}{N\left\{\begin{matrix}H\\H\\H\end{matrix}\right.}\qquad\underset{\text{[methylamine]}}{N\left\{\begin{matrix}H\\H\\CH_3\end{matrix}\right.}\qquad\underset{\text{[ethylamine]}}{N\left\{\begin{matrix}H\\H\\C_2H_5\end{matrix}\right.}$$

But one or both of the two remaining H's may be also replaced by methyl, ethyl, propyl, &c., and thus we obtain di-methylamine, di-ethylamine, di-propylamine, &c., and tri-methylamine, tri-ethylamine, tri-propylamine, &c., e.g.:—

$$\underset{\text{[ammonia]}}{N\left\{\begin{matrix}H\\H\\H\end{matrix}\right.}\qquad\underset{\text{[methylamine]}}{N\left\{\begin{matrix}H\\H\\CH_3\end{matrix}\right.}\qquad\underset{\text{[di-methylamine]}}{N\left\{\begin{matrix}H\\CH_3\\CH_3\end{matrix}\right.}\qquad\underset{\text{[tri-methylamine]}}{N\left\{\begin{matrix}CH_3\\CH_3\\CH_3\end{matrix}\right.}$$

The vegetable alkaloids are of similar structure; thus coniine is ammonia in which two atoms of hydrogen are replaced by the radicle C_4H_7, and nicotine is ammonia in which all three atoms are replaced by the triatomic radicle C_5H_7.

$$\underset{\text{[ammonia]}}{N\left\{\begin{matrix}H\\H\\H\end{matrix}\right.} \qquad \underset{\text{[coniine]}}{N\left\{\begin{matrix}C_4H_7\\C_4H_7\\H\end{matrix}\right. = C_8H_{15}N} \qquad \underset{\text{[nicotine]}}{2N(C_5H_7) = C_{10}H_{14}N_2}$$

In the alkaloids which contain oxygen the same process may be repeated in a more complicated manner.

It is important to note that the vegetable alkaloids are ammonia, not ammonium bases—that is, they combine with HCl without elimination of water as ammonia does; thus :—

$$\underset{\text{[ammonia]}}{NH_3} + \underset{\text{[hydrochloric acid]}}{HCl} = NH_3.HCl = \underset{\text{[ammonium chloride]}}{NH_4Cl}$$

$$\underset{\text{[morphine]}}{C_{17}H_{19}NO_3} + \underset{\text{[hydrochloric acid]}}{HCl} = C_{17}H_{19}NO_3.HCl = \underset{\text{[morphine hydrochloride]}}{C_{17}H_{20}NO_3Cl}$$

Some of the chief reactions of the alkaloids are :—

1. Alkaline reaction.

2. Insoluble in water. Soluble in acids with the formation of compounds, precipitated from such compounds by ammonia.

3. Some alkaloids are dextro-, some lævo-rotatory.

4. Sodium phosphomolybdate added to solutions acidified with nitric acid gives a yellowish precipitate.

5. Potassio-mercuric iodide (Mayer's solution) [1] produces flocculent yellowish white precipitates insoluble in acids and dilute alkalis, slightly soluble in excess of the reagent, easily soluble in alcohol, and generally also in ether.

6. Potassio-bismuthous, potassio-cadmic, potassio-platinic, and potassio-auric iodides also precipitate alkaloids.

7. Picric acid precipitates many alkaloids.

8. Tannin precipitates most alkaloids.

9. Platinic chloride, auric chloride, and many other reagents are also employed to precipitate alkaloids; individual members of the group differing in precipitability form the remainder.

10. Certain colour reactions are employed to identify many alkaloids, e.g. sulphuric acid gives a blood-red colour with thebaine, crimson with veratrine; nitric acid usually produces a yellow solution, but morphine and brucine give red; chlorine water and ammonia give

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[1] 13·5 grammes of mercuric chloride, and 49·8 grammes potassium iodide per litre. Mayer, *Liebig's Annalen*, cxxxiii. 236.

a green colour with quinine, red with narceine, and orange with narcotine, &c.

11. Many alkaloids may be identified by the temperature at which they sublime, and the microscopic character of the sublimate.

12. Many others are best identified by their physiological action, e.g. convulsions produced by strychnine, the peculiar taste sensations produced by aconitine, &c.

The occurrence and importance of alkaloidal substances in the animal body were first brought into prominence by a murder trial in Rome, in which a servant was accused of poisoning his master with the alkaloid delphinine. The accused was acquitted because the alkaloid obtained from the corpse, though giving many of the reactions of delphinine, differed from delphinine in certain other reactions of which the most important was its action on the frog's heart; delphinine brings the frog's heart to a standstill in diastole; the alkaloid obtained from the corpse stopped the heart in systole. The question was then taken up by a number of Italian investigators, of whom the most prominent was Francesco Selmi of Bologna. This investigator obtained from corpses which had undergone putrefactive changes, and also from various kinds of putrefying proteid (albumin, peptone, casein, &c.), a number of alkaloidal substances closely resembling the vegetable alkaloids both in reactions and physiological effects. He found some which, like atropine, dilated the pupil and accelerated the heart; others, like morphine, muscarine, strychnia, &c., in their physiological effects, and also in some of their chemical reactions and colour tests.

After Selmi's discoveries, other murder trials brought into prominence the subject of the cadaveric alkaloids in other countries; in London a criminal named Lamson was accused of murdering a young man with aconitine; the defence unsuccessfully set up was that the alkaloid found in the body of his victim was not a vegetable alkaloid at all, but one of the ptomaines produced by putrefactive processes after death.

The subject, however, has not merely a medico-legal interest, for it has been demonstrated that alkaloids exist in different forms of putrefying food.

In Germany sausages made with bad meat have been known to produce epidemics of a serious disorder, which we now know have been produced by these cadaveric alkaloids.

Certain forms of stale milk and cheese have caused more or less widespread outbreaks of serious morbid symptoms in those who have consumed these articles of diet. A ptomaine named tyrotoxicon by Dr. Vaughan[1] has been separated from bad cheese.

Poisoning by mussels and other forms of shell fish is also due to the presence in them of an animal alkaloid (mytilotoxine).

Of equal importance and interest to the physician are the poisons produced by bacteria in different forms of disease. Schmidt,[2] Panum,[3] and others separated from septic fluids a substance which was called *sepsin*; it was found to correspond closely in its reactions to the alkaloids; this was, however, before the general importance of the ptomaines was fully recognised. On injecting this material into the circulation, symptoms resembling those of septicæmia were produced. The conclusion was drawn, and probably correctly drawn, that this material was the substance produced by bacteria in ordinary septic processes, and that it is the real toxic agent in cases of blood-poisoning. Since then other ptomaines have been separated by more exact methods from pure cultivations of certain pathogenic bacteria, notably two named putrescine and cadaverine by Brieger; these are especially abundant in cultivations of Koch's comma or cholera bacillus; substances similar to these are probably the true chemical poisons in cholera.[4] There is still a large field open to investigators in this direction, but enough has been given in the way of instances to indicate the overwhelming importance of the subject to the pathologist. These ptomaines, then, are produced from animal substances by the influence of bacteria. The next questions which arise are, from what are the ptomaines formed, and how do the bacteria produce them? One of the most important of the ptomaines is neurine; this is a decomposition product of lecithin and doubtless arises from the lecithin contained in nerve, muscle, blood, and other parts of the body after death; also in eggs, milk, and cheese, and other forms of food. It is, however, fully proved that ordinary proteids will also, under the influence of certain bacterial growths, produce neurine, putrescine, cadaverine, as well as the simpler bases, such as methylamine, ethylamine, and ammonia. The question, how the bacteria do it? is a more difficult matter to answer. The decomposition of lecithin produced by these organised ferments is no doubt similar to that brought about by ordinary chemical reagents; but we cannot describe the decomposition of proteids until we know their composition. The different views now held as to the constitution of a proteid will each involve a separate theory as to how an alkaloid may be formed from it. It may, however, be regarded as settled that animal alkaloids, whether of the ptomaine or leucomaine series, are produced anaërobically (*see* p. 164).

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[1] *Zeit. physiol. Chem.* x. 146.

[2] *Inaug. Diss. Dorpat*, 1869.

[3] *Virchow's Archiv*, 1863, vol. xxvii. p. 240; vols. xxviii. xxix. and others.

[4] The probability that cholera is caused by an alkaloid was first pointed out by Lauder Brunton (*Brit. Ass. Reports*, 1873); he deduced it from the similarity of the symptoms of cholera and alkaloid (muscarine) poisoning. Cadaverine and putrescine are not markedly toxic.

The priority of describing an alkaloidal substance in animals is claimed by Dupré and Bence Jones. These observers in the year 1866 described an alkaloidal substance, which they separated from the solid and liquid tissues of animals, and they named it 'Animal Quinoidine.'[1] The honour is also claimed for a chemist who worked at Stettin, named Marquardt,[2] who described an alkaloid obtained from a corpse, to which he gave the name 'septicin,' and which he found was similar in its action to coniine. The work of Panum, Schmiedeberg, Bergmann, and Schmidt on sepsin has been already alluded to. These were all more or less gropings in the dark until the master hand of Selmi[3] placed the matter on a satisfactory basis; it was he, too, who invented the word 'ptomaine.' The details of methods of separation and of analysis have, as usual, been left to a multitude of German workers, but Brieger stands head and shoulders above all the rest. In France the subject has been taken up by Gautier, who has produced numerous memoirs on the subject.[4]

Brieger was the first to obtain from the uncrystallisable extracts and syrupy products of previous investigators pure materials in a crystalline form. He found it necessary to adopt considerable modifications of the Stas-Otto process, which is the best for the extraction of the vegetable alkaloids. The pure crystalline alkaloids were not only analysed by him, but in many cases their constitution was worked out also. He found that the bases isolated from putrefactive mixtures were less poisonous than those produced by pathogenic bacteria. These latter poisons include such substances as typhotoxine (from cases of typhoid fever), tetanine[5] (from cases of tetanus), and several others; on account of their powerful poisonous properties Brieger has separated them from the other ptomaines, and calls them *toxines*.

It has been recognised that it is very difficult to draw the limits of the word ptomaine; the products of metabolism of bacteria are not, in general, different from those of the higher organisms; thus choline, neurine, creatinine, &c., are normal products occurring in, or separable from, healthy animal tissues. Gautier has invented the word *leucomaine* for the basic products produced in the tissues of living animals by metabolic processes, while he reserves the word ptomaine for those formed by putrefaction after death. It must, however, be remembered that many leucomaines are powerful poisons.

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[1] *Proc. Roy. Soc.* xv. 73. *Zeit. f. Chem.* 1866, p. 348.

[2] Schuchardt in *Maschka's Handb. d. ger. Med.* ii. 60.

[3] *Deutsch. chem. Gesell.* xi. 808.

[4] For the latest see *Bull. Soc. Chim.* xi. 6.

[5] For the last paper on tetanine from a case of tetanus, *see* Brieger, *Berlin. klin. Wochenschrift*, 1888, No. 17.

If ptomaines and leucomaines are to include all bases produced in animals, the simpler substances, like methylamine, trimethylamine, &c., must all come under either one or the other heading. This is especially necessary, since it has been shown that probably the diamines, like putrescine[1] and cadaverine,[2] are derived by oxidation from the monamines.[3]

Such, then, is a sketch of the ptomaines, in which their importance has been indicated by a few examples. The subject is yet in its infancy, and many more facts must be collected before positive general conclusions can be drawn. Without at all wishing to minimise their importance, it is, however, necessary to insist on one point, and that is that all poisons produced by bacteria are not necessarily ptomaines, that all mysterious symptoms in obscure complaints cannot be as yet attributed to leucomaines.

There is always a tendency after any great discovery is made to attribute to it wider importance than it really possesses. We have many instances of this in pathology : the doctrine of the solidists, which totally excluded humoralism, was an outgrowth of Schwann's great generalisation we call the cell theory. Similarly there can be little doubt that under the influence of the germ theory many premature conclusions were jumped at, concerning the association of organisms with disease. Ptomaines are now displacing somewhat the microbe, which was formerly regarded as all-important, but this must not be pushed too far. The discovery of ptomaines is complemental, not antagonistic, to the germ theory. We must remember that there are many powerful poisons which are not alkaloids at all. Snake poison is a striking example of this; it is a poisonous proteid indistinguishable from other proteids by its reactions. The products of digestion produced normally in the alimentary canal (albumoses and peptones) are also powerful poisons. Recently it has been surmised that the bacillus anthracis produces a poisonous albumose, which also has the power of conferring subsequent immunity from the attacks of the bacillus.[1] Not doubt the poisonous proteids, as well as the ptomaines, will have to be very largely reckoned with in the investigations of the poisons of diseases.

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[1] Putrescine ($C_4H_{12}N_2$) is chemically tetramethylenediamine (*Ber. deutsch. chem. Gesell.* xxi. 2938).

[2] Cadaverine ($C_5H_{14}N_2$) is pentamethylenediamine (Ladenburg, *Ibid.* xix. 2585). Two other alkaloids named neuridine and saprine have been separated by Brieger, which are isomeric with cadaverine.

[3] A full explanation will be found in an interesting paper by Baumann and v. Udranszky (*Zeit. physiol. Chem.* xiii. 562). These observers show that the two diamines which are found in cholera, and in pure cultivations of the cholera bacillus, are also found in the urine and fæces of patients suffering from cystinuria, a condition apparently very different from cholera. Normal urine is toxic, but this is probably due to the inorganic potassium salts in it (Stadthagen, *Zeit. klin. Med.* xv. parts 5 and 6), not to any alkaloid. Ptomaines have never (until these cases of cystinuria were described) been satisfactorily demonstrated to exist either in normal or pathological urine, though theoretically their presence there is possible, for the ptomaines formed by putrefaction in the intestine might conceivably be partly reabsorbed and then excreted in the urine. Ponchet (*Compt. rend.* xcviii. 1560) has stated that normal urines contain poisonous alkaloids; his methods and results are, however, full of fallacies. Hunter has found diamines in the urine in cases of pernicious anæmia.

METHODS OF SEPARATION OF PTOMAINES

The first method of any importance for the separation of alkaloids from organic mixtures was proposed by Stas,[2] subsequently modified by Otto,[3] and now known as the Stas-Otto process. Other methods have been introduced by Dragendorff,[4] Sonnenschein,[5] Selmi,[6] and Brieger.[7] The last-named observer's process is specially adapted for the separation of ptomaines.

The Stas-Otto process.[8]—The substance to be operated on, if solid, is finely divided, and repeatedly digested for many hours with fresh quantities of rectified spirit at a temperature of 55° C. Liquids are also treated with twice their volume of spirit. The residue is finally digested at 35° C. with spirit faintly acidified with acetic acid; it is then once or twice more digested with unacidified spirit. The alcoholic liquids obtained before acidification are mixed together, and rapidly and momentarily raised to 70° C., cooled, and filtered; those obtained with and after the use of acetic acid are mixed together and similarly treated. But the two liquids, the unacidified and the acidified, are not mixed with one another till later. Each infusion is then concentrated to a syrupy consistency at a temperature of 35° C. To the syrup about 30 c.c. of absolute alcohol are added, with constant stirring and grinding in a mortar. The alcohol is poured off from the pasty mass, and replaced by successive portions of 15 c.c. of alcohol, so long as a colour is imparted to it. The alcoholic extracts are mixed, filtered, and the filtrate concentrated as before at 35° C. We have again a syrupy residue, both from the unacidified and the acidified original extracts; each is diluted with water, filtered, and the filtrates mixed. They should now measure 15–20 c.c. This is partially neutralised with soda, but, still faintly acid, is placed into a well-stoppered tube. This liquid contains all the alkaloids present in the original material, and is free from proteids. This aqueous liquid (A) is now covered with twice its volume of ether, and the whole is mixed by gently and repeatedly inverting the tube. The ether is allowed to separate, and is pipetted off. A fresh quantity of ether is then used, and the extraction with ether repeated until a few drops on evaporation leave no residue; four or five extractions generally suffice. Each ethereal solution is washed by shaking it with 5 c.c. of water to which a drop of sulphuric acid has been added: they are then mixed, and on evaporation may leave an oily residue, which should be reserved for further examination. The bulk of the alkaloids, however, remain insoluble in ether. The acid liquid (A) (after washing with ether), and the acidulated water used in washing the ether extracts, are mixed, made alkaline with sodium carbonate, and exhausted, once with a mixture of chloroform and ether (1 : 3), and subsequently three or four times with ether alone. These extracts are successively washed with water, then acidulated water, and lastly water again. The alkaloids are thus first liberated by the alkali, then dissolved in the ether-chloroform, then again converted into sulphates, which, being insoluble in ether and chloroform, pass into the acid solutions, impurities being left behind in the ether. The acid liquid and the final wash water are mixed, washed with ether, once more made alkaline, and again extracted with chloroform-ether and ether. These extracts are washed with water, made barely alkaline with sodium carbonate, filtered, and the filtrates evaporated to dryness below 35° C. This may be then dried at 100°, cooled and weighed, the weight being that of the total alkaloids. If a volatile alkaloid is present, the residue will be oily; whether these are present should be discovered by first evaporating a few c.c. only. If they do occur, the extracts must be acidified with hydrochloric acid, and then on evaporation the non-volatile hydrochlorides are left. The free alkaloids obtained in the first instance are converted into hydrochlorides, dissolved in water, and then separated and tested for according to their various properties. They are best separated by the use of different solvents (petroleum ether, benzene, chloroform, alcohol, &c.), in which some are and some not soluble.

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[1] *See* p. 168.

[2] Stas, *Liebig's Annalen*, lxxxiv. 379.

[3] Otto, *Ibid.* c. 39.

[4] Dragendorff, *Gerichtl. chem. Ermit. v. Gift*, 1876.

[5] Sonnenschein, *Liebig's Ann.* cv. 45.

[6] Selmi, *Journ. of Chem. Society*, 1877, p. 93.

[7] Brieger, *Die Ptomaïne*, part i. 1885; part ii. 1885; part iii. 1886.

[8] As modified by Dr. Stevenson, *Watts' Dict.* vol. i. 1888. *Art.* 'Alkaloids poisonous.'

Brieger's method.—The mass of putrefying material is boiled with water, filtered, and the filtrate precipitated with subacetate of lead. This precipitate is filtered off; a stream of hydrogen sulphide is passed through the filtrate, and the lead sulphide separated by filtration. The filtrate is evaporated to a thin syrup, and this is extracted with amyl alcohol. The extract is repeatedly treated with water, and then concentrated, then made strongly acid with sulphuric acid, and repeatedly shaken with ether, which removes oxy-acids. Freed from ether it is evaporated to a quarter of its bulk, and thus volatile fatty acids are driven off. The sulphuric acid is precipitated by baryta, and the precipitate removed by filtration: the excess of baryta is precipitated by a stream of carbonic acid, and this is also removed by filtration. The fluid is warmed for some time on the water-bath, cooled, and precipitated with mercuric chloride. The precipitate is well washed and decomposed by sulphuretted hydrogen; the mercuric sulphide is filtered off, and the filtrate concentrated. Inorganic substances crystallise out first, which are filtered off and washed with absolute alcohol. Subsequently long needles form, of organic nature, which are soluble in water and dilute alcohol, but insoluble in absolute alcohol, ether, benzene, and chloroform. These substances consist of the ptomaines, and they are then separated by fractional precipitation with the chlorides of platinum or gold. Baumann and v. Udranszky have separated cadaverine and putrescine by the different solubilities in ether and alcohol of their benzoyl-compounds.[1]

In some of his researches Brieger has shortened the procedure by precipitating the putrid fluids after boiling and filtering directly with mercuric chloride, i.e. the first precipitation, that with lead acetate, is omitted. As mercuric chloride does not precipitate all alkaloids, both precipitate and filtrate must be examined.

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[1] Baumann and v. Udranszky, *Zeit. physiol. Chem.* xiii. 562.

GENERAL PROPERTIES OF THE ANIMAL ALKALOIDS

The animal like the vegetable alkaloids may be divided into two groups: those which do, and those which do not contain oxygen. Those which do not contain oxygen are the ptomaines proper, and, like the non-oxygenated vegetable alkaloids, are liquid, volatile, and odorous. The oxygenated alkaloids are crystalline and fixed.

They all have an alkaline reaction.

They are oxidisable and unstable, especially under the influence of an excess of mineral acid, which colours them red, and then converts them into a resinous mass.

Their chloroplatinates and chloroaurates vary much in solubility.

Picric acid precipitates most of them, the colour of the precipitate usually being pale yellow.

Tannin, mercuric chloride, &c., also produce insoluble precipitates as a rule.

Phosphomolybdic acid precipitates all the alkaloids.

The ptomaines are energetic reducing agents, decomposing chromic acid, iodic acid, and silver nitrate. With ferricyanide of potassium and ferric chloride they give Prussian blue. This was at one time considered to be characteristic of the animal alkaloids, but it has been found that many vegetable alkaloids give the same test, and a few of the animal alkaloids (especially those containing oxygen—Brieger) do not give it. There is, so far as is at present known, no class reaction by which the alkaloids of animal can be separated from those of vegetable origin.

ENUMERATION OF THE ANIMAL ALKALOIDS

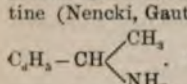
The animal alkaloids which up to the present have been obtained in a pure condition may be arranged as follows:—

1. Non-oxygenous ptomaines—
   - Hydro-collidine
   - Collidine
   - Parvoline
   - Neuridine
   - Saprine
   - Cadaverine
   - Putrescine
   - Mydaleine
2. Oxygenous ptomaines—
   - Neurine
   - Choline
   - Muscarine
   - Gadinine
   - Mytilotoxine
   - Tetanine
   - Typhotoxine
3. Leucomaines of the uric acid group—
   - Carnine
   - Adenine
   - Guanine
   - Sarcine or Hypoxanthine
   - Xanthine
   - Pseudo-xanthine
4. Leucomaines of the creatinine group—
   - Creatinine
   - Xantho-creatinine
   - Cruso-creatinine
   - Amphi-creatinine

1. **The non-oxygenous Ptomaines** are liquid and volatile. The properties of the chief members of the group are:—

a. *Parvoline*, $C_9H_{13}N$. This was first separated from the putrid flesh of the mackerel and horse. It is an oily base, of a yellow colour, boiling a little below 200° C. Its chloroaurate and chloroplatinate have been prepared. These are crystalline, and the latter is the more insoluble of the two (Gautier and Etard).

b. *Hydro-collidine*, $C_8H_{13}N$ (boiling-point 210° C.), and *Collidine*, $C_8H_{11}N$; the first-named of these two other bases derived from the same source as the preceding, the latter from the putrefied products of ox pancreas and gelatine (Nencki, Gautier). Nencki considers collidine to be iso-phenyl-ethylamine,



$$C_6H_5-CH\left\langle\begin{matrix}CH_3\\NH_2\end{matrix}\right..$$

These three bases are all highly toxic.

c. *Neuridine*, $C_5H_{14}N_2$, is a constant product of the putrefaction of proteids. Its hydrochloride, platino-chloride, and auro-chloride have been crystallised and analysed, but the free base is so unstable that it has never been obtained pure. A solution of sodium hydrate breaks up the hydrochloride of neuridine into dimethylamine and trimethylamine (Brieger).

d. *Saprine*, $C_5H_{14}N_2$, though isomeric with the preceding, differs from it in the solubilities of its salts, and probably also in chemical constitution.

e. *Cadaverine*, $C_5H_{14}N_2$, a third isomeride, generally appears late in ordinary putrefactive processes, but readily in cultivations of the cholera bacillus and Finkler-Prior vibrio. Its chemical constitution has been worked out by Ladenberg,[1] who has found that it belongs to the group of diamines, and that it is penta-methylene-diamine. It has a spermatic odour, and boils at 115°–128° C.

f. *Putrescine*, $C_4H_{12}N_2$, is also a diamine, being tetra-methylene diamine. It is usually found accompanying cadaverine, but makes its appearance rather later. The chief work on this diamine has been done by Brieger,[2] Bœklisch,[3] and its constitution was discovered by Baumann and v. Udranszky.[4] These two diamines also are found in the fæces and urine in cases of cystinuria (Brieger and Stadthagen,[5] Baumann, and v. Udranszky[6]).

They are both poisons, but not virulent ones, and the symptoms they produce are very similar to some of those of cholera (hæmorrhages and necrosis),[7] but the muscular cramps and other prominent symptoms of that disease are probably produced by other poisonous alkaloids (toxines, as Brieger would call them) not yet separated.

Other poisonous alkaloids belonging to this group have not yet been fully examined; thus a base named *mydaleine* was described by Brieger, and this probably is also a diamine; it is markedly toxic.[8]

2. **The oxygenous Ptomaines** may in many cases be obtained from healthy tissues; and they are also formed from those tissues on the occurrence of putre-

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[1] *Ber. deutsch. chem. Ges.* xix. 2585. The original formula assigned to cadaverine by Brieger was $C_5H_{16}N_2$.

[2] *Berlin. klin. Woch.* 1887, No. 44.

[3] Bœklisch, *Ber. d. Gesell.* xx. 1441.

[4] *Ibid.* xxi. 2938.

[5] *Arch. path. Anat.* cxv. part 3.

[6] *Zeit. physiol. Chem.* xiii. 562.

[7] For symptoms *see* v. Behring, *Deutsch. med. Wochensch.* xiv. No. 24. Scheuerling, *Maly's Jahresb.* 1887, 491. Fehleisen and Grawitz, *Virchow's Arch.* cx. 1.

[8] See Articles by Lauder Brunton on Food and Poison, *Practitioner*, vol. xxxv. Aug. Sept. and Oct. 1885.

faction. They may thus be in many cases leucomaines as well. It is very doubtful if they exist in a free state in healthy tissues; probably they are formed by the action of the reagents used on analysis, e.g. the neurine is derived from lecithin (*see* Chap. XXIV).

a. ***Neurine***, $C_5H_{13}NO$, is trimethyl-vinyl ammonium hydroxide. This is a syrupy base and strongly alkaline; its chloroplatinate crystallises readily. It is a constant product of cadaveric putrefaction, and a more powerful toxic agent than the alkaloid choline found with it.

b. ***Choline***, $C_5H_{15}NO_2$ (= neurine + water), is trimethyloxyethylene-ammonium hydroxide. This substance is very similar in its properties to neurine. A full account of it will be found in the chapter on the nervous tissues. Choline is generally also called neurine, but Brieger restricts the latter word to the closely related alkaloid just described.

c. ***Muscarine***, $C_5H_{15}NO_3$, was discovered by Schmiedeberg and Koppe[1] in the poisonous mushroom -*agaricus muscarius*. Schmiedeberg and Harnack[2] also obtained it from choline by the oxidising action of nitric acid—2 atoms of the hydrogen of the choline being removed by the nitric acid from the choline; muscarine is thus similar in constitution to the aldehydes. Brieger has found the same substance in putrid fish.

These three substances are all powerful poisons; neurine and choline acting like curare on the end-plates; muscarine on the muscular tissue itself, especially of the heart. All three are antagonistic to atropine so far as relates to its action on the heart and glandular system.

d. ***Gadinine***, $C_7H_{16}NO_2$, was obtained by Brieger, mixed with muscarine from putrefying cod-fish. It is, however, less toxic than muscarine.

e. ***Mytilotoxine***, $C_6H_{15}NO_2$, is the active agent in mussel-poisoning (Brieger).

f. ***Typhotoxine***, $C_7H_{17}NO_2$, is an alkaloid obtained from pure cultures of the typhoid bacillus, and is regarded by Brieger as the chemical poison in typhoid fever.

g. ***Tetanine***, $C_{13}H_{22}N_2O_4$, is the supposed toxine in cases of tetanus (Brieger).

3. **Leucomaines of the uric acid group.**—The substances enumerated under this heading have been already described with the uric acid group (*see* p. 90), with the exception of the last named, pseudoxanthine, to which Gautier ascribes the formula $C_4H_5N_5O$.

4. **Leucomaines of the Creatinine group.**—Of these creatinine has been described in connection with creatine, one of the amido-acids (*see* p. 84). Xanthocreatinine, $C_5H_{10}N_4O$, crusocreatinine, $C_5H_8N_4O$, amphicreatinine, $C_9H_{19}N_7O_4$, are bases which have been separated from muscle, together with pseudoxanthine by Gautier. They are all poisonous.

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[1] *Das Muscarin*, Leipzig, 1869.

[2] *Arch. exp. Path. u. Pharmak.* vi. 101.

PART III

THE TISSUES AND ORGANS OF THE BODY

CHAPTER XIV

THE CELL

THE cell is the structural unit of living things. It consists of a mass of material which has a jelly-like consistency, and possesses the powers of movement, assimilation and the like, which are known as vital. This living substance, or organic basis of life, is known as *protoplasm*. Within the protoplasm are minute granules of various kinds, and an important structure of more solid consistency, known as the *nucleus*. Outside the protoplasm there is in some cases, especially in vegetable cells, an investing membrane, known as the *cell-wall*. The cell-wall, however, is not essential, and in animal cells is generally absent.

The lowest animals with which we are acquainted consist of single cells, and are called unicellular.

The lowest plants are also unicellular.

The highest animals and plants are also originally unicellular; the human ovum is, for instance, a typical cell. The development *ab ovo* is termed the life-history of an organism (ontogeny), and this development is, according to the Darwinian hypothesis, in its essential features similar to the historical development (phylogeny) of the higher organisms from simpler and ultimately from unicellular forms, which has occupied untold ages in the past.

Physiology may be described as the science which treats of the functions of protoplasm and its modifications. Some of these functions can be accounted for by chemical laws; this constitutes the department of the science known as Chemical Physiology, or Physiological Chemistry; other functions are physical manifestations; the greater our advance of knowledge of protoplasm becomes, the more does it become evident that all vital phenomena may be classed under one or other of these two heads; the unexplained residue we must classify as vital, using that word simply for want of a better, and not as implying any belief in the existence of a special or vital force.

If we take a single animal cell, either a unicellular organism like an Amœba, or a white blood corpuscle, which is an instance of a cell retaining its primitive structure in the adult form of higher animals, we find that it has the following properties :

1. *Power of movement.*—The shape of the cell is continually changing, processes being extended and withdrawn. Movement may be sometimes apparently spontaneous, but usually it is excited by the influence of external agencies—heat, foreign particles, &c. These agencies are termed *stimuli*, and the power of responding to a stimulus by contraction is known as *irritability*.

2. *Power of assimilation*, that is, of absorbing dead matter, and converting it by chemical changes into a part of itself, i.e. into protoplasm or living matter.

3. *Power of growth* ; this follows from the last.

4. *Power of secretion* ; or the elaboration from protoplasm of new substances ; this is seen in the formation of vacuoles, but to a greater extent in the higher animals, where certain cells, in organs called secreting glands, are set apart specially, for the formation of highly elaborate materials, known as enzymes or ferments. Some secretions are merely discharged from the cells as waste products ; these are known as excretions.

5. *Power of reproduction*, or the giving off of living things similar to themselves ; in the simplest condition this is brought about by *budding*—the detachment of minute particles of protoplasm which grow into adult cells ; or by *fission*, due to the splitting of the cell, including its nucleus, into two, each daughter cell growing into an adult, which in its turn undergoes a similar division.

In the development of a higher animal from a single cell, or ovum, there is, after fertilisation, first a division of that cell into two, each of which again divides, so that four, and then by a similar process eight, sixteen, and so on, cells are formed. The cells so formed do not become detached from one another, but remain adherent, so that a little mass of simple cells, each like the original, is formed. These become arranged in the form of a little sphere, at first solid, and then containing liquid shed out from the cells ; a little later it will be found that the layers of cells are three deep ; the outermost layer is termed the *epiblast* or *ectoderm*, the innermost the *hypoblast* or *endoderm*, or *entoderm*, while the middle one is the *mesoblast* or *mesoderm*. From these three layers all the tissues and organs of the adult are formed ; the epidermis and nervous system from the epiblast ; the lining membrane of the alimentary and respiratory cavities, with the cells of the digestive glands, from the hypoblast, and the rest of the body from the mesoblast.

In the further development of the adult from the three primary embryonic layers, there is not merely subdivision of the cells, but the cells in certain parts become modified or altered from their primitive condition; some become hollow and adherent to one another to form blood vessels; others become elongated and thread-like, to form muscular fibres; in other parts the cells become modified chemically, as in the horny layers of the epidermis, or the mucin-yielding cells of salivary glands. In other parts, as in the connective tissues, the cells may become separated by an intercellular substance, in which fibres may form, or in which, as in bone, calcareous matter may be deposited. These are merely instances of the variations that may occur. In the chapters that follow this, the several tissues and organs so formed, will be taken *seriatim.* For the present we have more especially to deal with the structure of the primitive cell. Chemical investigation of such an object is fraught with difficulties, and must in many cases be performed on a microscopic slide; in certain other cases, however, as with pus cells, liver cells, &c., it is possible to obtain large collections of cells, and then the methods of macro-chemistry can be applied.

The cell theory is associated in greatest measure with the name of Theodor Schwann; and I translate here the following sentences from the life of that scientist written by Léon Fredericq of Liège.[1]

'Previous to Schwann's time, it was known that in animals there were example of organs formed of cells. Müller had described them in the spinal cord; Henle had studied them in the epidermis; Henle and Purkinje in glands; Ehrenberg and Valentin in nerve centres, &c. But these were isolated facts, and indeed certain *savants* looked upon them as exceptions. No one had yet thought of carrying into the domain of animal histology the general notions derived from the microscopic study of vegetable structures. Schwann has himself told the accident which gave him the first idea in his great discovery.

'"One day I was dining with M. Schleiden, the illustrious botanist, and he was telling me of the important part played by the nucleus in the development of vegetable cells. I suddenly remembered having noticed a similar appearance in the cells of the spinal cord, and the same moment I grasped the importance of being able to show that this nucleus plays a part, similar to that observed in plants."

'The two scientists immediately repaired to the anatomical theatre to examine the nuclei in question, and Schleiden recognised their perfect resemblance to vegetable nuclei.

'"Since that moment," Schwann continues, "all my efforts tended to try to prove the pre-existence of the nucleus in the cell.

'"Once arrived at a satisfactory conclusion concerning the cells of the spinal cord, and of cartilage, the origin of the elementary parts of other tissues by the same mode of development, that is to say, by means of cells, was no longer a matter of doubt to me, and further observation has entirely confirmed this view. The microscope has shown me that all the varied forms in the animal tissues are nothing but transformed cells, that uniformity of texture is found throughout the animal kingdom, and that in consequence a cellular origin is common to all living things. All my work has authorised me to apply to animals as to plants the doctrine of the individuality of cells."'

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[1] Liège, 1884.

The first matter in connection with cells that we shall take up is one which is not strictly a chemical one, but one which can nevertheless not be omitted in a consideration of the physiology of cells; this is the physiology of protoplasmic movement.

PROTOPLASMIC MOVEMENT

This section is very largely an abstract of Engelmann's article on this subject in Hermann's Handwörterbuch der Physiologie.[1]

The movement of living protoplasm must be classed with muscular and ciliary movement, with which it is closely connected by numerous transitional forms, as phenomena of contractility.

The special character of protoplasmic movement lies in this, that the particles of the contractile mass move, not in relation to any fixed position of equilibrium, but as do the moving particles of a fluid.

Transitional forms of movement between this and the highly ordered and limited contraction of a muscle occur, and of these the following are instances: the movements of the tentacles of acinetæ,[2] the superficial sarcode of sponges,[3] the endothelial cells of young blood capillaries,[4] the pigment cells of amphibians and reptiles,[5] and many others.

The oldest description of a protoplasmic movement is that by von Rosenhof (Monatlich herausg. Insectenbelustigung, 3ter Th. p. 621. Nürnberg, 1755) of a fresh-water Amœba.

Twenty years later came Corti's (Lucca, 1774) description of the rotation of the cell sap in Chara. In the early part of the present century the wide distribution of this phenomenon in vegetables was demonstrated by Meyer,[6] R. Brown,[7] Amici and others. Then came Dujardin's[8] description of the movements of the body substance of certain rhizopod animals. This substance he termed *sarcode*. The movement of white blood corpuscles was first observed by Wharton Jones in 1846.[1] V. Mohl[2] gave the name *protoplasm* to the motile substance in plant cells, and Cohn[3] first advanced the suggestion that this and sarcode were identical. The actual identity of animal and vegetable protoplasm was more clearly proved by Max Schultze,[4] de Bary,[5] Haeckel,[6] Kühne[7] and others ; and a more complete knowledge concerning its movement has been afforded by Nägeli, Brücke, and Heidenhain. The wandering of amœboid cells in animal tissues was brought into general notice by v. Recklinghausen,[8] and the importance of this in physiological and pathological process was shown by Stricker and Cohnheim.

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[1] Translated by A. G. Bourne, D. Sc.: *Quart. Journal Micr. Sci.* xxiv. 369.

[2] Lieberkühn, *Bewegungerschein. d. Zellen*, p. 346. Marburg, 1870.

[3] Stricker, *Wiener Sitzungsb. d. Math. naturw. Cl.* lxxiv. p. 313, 1877, and others.

[4] G. Seidlitz, *Beiträge zur Descendenztheorie*, p. 31–36, Leipzig, 1876.

[5] Hering, *Cent. f. med. Wissens.* 1869, No. 4, p. 49.

[6] In Vallisneria, 1827.

[7] In Tradescantia, 1830.

[8] *Bull. de la soc. des sci. nat. de France*, 1835, No. 3. *Ann. sci. nat.* iii. 2nd sér. p. 312, 1835. iv. p. 343, 1835.

The movements of naked protoplasm may be distinguished into three types—amœboid, streaming, and gliding. *Amœboid movement* shows itself in the protrusion and retraction of conical and at first generally hyaline processes, into which the granules from the interior stream in and out. The processes may ramify and even form networks. If the processes fasten themselves to fixed bodies, they can by shortening draw the rest of the protoplasm after them, and so produce a movement of translation. These movements may be readily seen in white blood corpuscles, in many unicellular animals, in numerous ova (hydra, sponges, &c.), in connective tissue cells, and in the plasmodium of myxomycetes, where the movements are visible to the naked eye. *Streaming movement* occurs in many protozoa (Heliozoa, Radiolaria, &c.). Out of the protoplasmic body long thin threads of protoplasm spring, and upon their surface a great number of fine granules in active streaming movement are seen, the main substance of the threads themselves often showing no movement, or only slow changes of form. *Gliding movement* : in this case, extremely thin layers of protoplasm devoid of granules move along outside a firm cell wall, and by means of this movement the whole body progresses over a firm substance in a gliding or creeping manner. The rapidity of the movement seldom exceeds 0·04 mm. in a second. This form of movement is well seen in the diatoms.

The movements of cells bounded by firm integuments.—This case is chiefly realised in vegetable cells, and botanists distinguish two varieties : (1) *Circulation*, in which contractile protoplasmic threads stretch inwards from the cell wall, traversing the cell space, which is filled with fluid; these threads divide, fuse, form sheets and generally exhibit streaming granules. These movements are well seen in the staminal hairs from Tradescantia; and in the animal kingdoms in Noctiluca, tentacles of medusæ, gill fibres of Branchiomma, &c. (2) *Rotation*: here the protoplasm lining the cell walls rotates as a connected mass round the interior of the cell, generally following constant tracks and with an even velocity; chlorophyll grains, crystals, nuclei, &c., are carried with it. This is well seen in many vegetable cells like Vallisneria, and here also must be classed the rotation of the endoplasm of Paramœcium and Vorticella.

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[1] *Proc. Roy. Soc.* 1846.

[2] *Bot. Zeitung*, p. 73, 1846.

[3] *Nova Acta Leop. Caes.* xxii. 2. p. 605, 1850.

[4] *Arch. f. Anat. u. Physiol.* 1858, p. 330 ; 1861, p. 1.

[5] *Zeit. f. wiss. Zool.* x. p. 88.

[6] *Die Radiolarien*, Berlin, 1862. *Zeit. wiss. Zool.* xv. p. 342, &c.

[7] *Unters. ü. das Protoplasma*, Leipzig, 1864. *Arch. f. Anat. u. Physiol.* 1859, p. 564.

[8] *Arch. f. path. Anat.* xxviii. p. 157, 1863.

General conditions of protoplasmic movement.—(*a*) *Temperature.* —Speaking generally, the movement ceases below 0°C. and above 40°C. Within these limits the velocity of the movement increases with the temperature. The optimum temperature is generally a few degrees below the maximum temperature compatible with movement. When warmed to the maximum, naked cells become spherical. When subsequently cooled the protoplasm does not resume movement, as the contained proteids have been coagulated by the heat, and the protoplasm is dead. When protoplasm enters suddenly into *heat-rigor*, as by a jet of steam playing upon it, it has no time to change its form, but remains in the position it had the moment before death. A low temperature on the other hand, though it stops movement, does not kill the protoplasm; even after actual freezing, protoplasm will when thawed resume movement. Kühne lowered Tradescantia hairs to −14°C. for five minutes, and after careful thawing the threads were again found in active streaming movement. Animal life in its simplest form seems to withstand great cold without apparent injury; thus McKendrick and Coleman[1] exposed bacterial spores to a temperature of −83° for 100 hours, without succeeding in killing them.

(*b*) *Imbibition water* acts like a degree of temperature. There is a maximum (over 90 per cent.) and a minimum (below 60 per cent.) for the amount of contained imbibition water at which movements stop. When the maximum is gradually approached, the protoplasmic mass becomes spherical; removal of the excess of water with indifferent substances like saline solutions often cause the movements to be reinduced after even some minutes of *water-rigor*. The withdrawal of water produces a temporary or permanent *dry-rigor*. Lower organisms like spores, encysted amœbæ, &c., may be dried and kept for years in this condition; after that time on the application of moisture they resume activity.

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[1] *Proc. Royal Instit. of Great Britain*, May 29, 1885.

(*c*) *Oxygen.*—Withdrawal of oxygen ultimately produces death, but in media free from oxygen, protoplasmic movement will continue for some hours, the cells giving off carbonic anhydride. The oxygen previously taken into the cell is in a state of loose combination; when however this storage oxygen is exhausted the cell dies.

(*d*) *Poisons.*—A slight excess of acid, and a rather larger quantity of alkali, causes a cessation of protoplasmic movement, which can be counteracted for a time by neutralisation. A very weak alkali stimulates the movement. Carbonic acid gas, ether, and chloroform vapour stop it. Veratrine (Kühne) and quinine (Binz)[1] act similarly.

(*e*) *Artificial stimulation.*—The following may be used as stimuli to movement: weak electrical currents, or sudden alterations of temperature, within the temperature range of contractility; in the case of most cells, light does not act as a stimulus; in other cases it does; for instance the Pelomyxa moves actively in the dark, and becomes spherical when exposed to light; plant cells containing chlorophyll are most susceptible to the influence of light. Mechanical stimuli like pressing, bruising, tearing, &c., and chemical stimuli like ammonia vapour, various strengths of saline solutions, &c., may also be employed; as a rule, however, in chemical stimulation, accessory phenomena like shrinking, swelling, or coagulation interrupt and mask the effect of the excitation.

Theoretical conclusions.—Protoplasm must be regarded as an aggregate of exceedingly minute, contractile, excitable form-elements, and the movement as a whole is the result of the changes in form of these very small elements. The nature and cause of the changes in form of the latter remains provisionally undetermined.

With regard to their form we may take it for granted that when in a condition of maximal excitation they are almost spherical, and when not excited are generally elongated or thread-like.

The mechanical behaviour of naked protoplasm teaches us that the changes in form must take place with a force which exceeds, as a rule, the force which the elements, if they were fluid, would put forth, in order to assume a spherical form.

These contractile elements may be called 'Inotagmata.' Probably they are positive uniaxial doubly refracting.[2]

The active as well as the passive phenomena of protoplasmic movement compel us further to make the assumption that the inotagmata of protoplasm are not like those of muscles and cilia arranged in a relatively firm manner with their axes in one definite direction, but are fastened together loosely and are capable of moving one against the other in all directions ; still the possibility of a temporary or permanent grouping of a greater or less number of inotagmata into definitely shaped larger masses (fibres, networks, membranes, &c.) is not excluded.

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[1] *Arch. mikr. Anat.* iii. p. 383, 1867.

[2] Contractilität und Doppelbrechung, *Pflüger's Arch.* xi. 1875.

As a reason for the possibility of alteration of arrangement of the protoplasmic particles, and in connection with the prevailing views concerning the molecular structure of organised masses, we must assume the existence of a capability for the imbibition of important quantities of water between the inotagmata, and the larger masses or inotagma groups. The motility, as already shown, increases or diminishes with the quantity of this water.

PHYSICAL AND CHEMICAL PROPERTIES OF PROTOPLASM

Engelmann[1] describes contractile protoplasm as a homogeneous, transparent, almost always colourless mass, with a higher refractive index than water, but lower than oil. In some cases where it has the form of fibres or thin layers with a prevailing movement in one direction, it is doubly refracting, and as in muscles and cilia with a single positive axis, the optical axis coincides with the direction of the movement.

Different portions of the same protoplasmic mass may have different refractive powers, and during movements the refractive power of the same portion changes to a considerable extent.

Protoplasm is semifluid, does not mix, but swells up with water ; it is cohesive and extensible. Though the superficial layers of many cells are firmer than the interior, a distinct membrane is absent as a rule in animal cells.

Protoplasm, almost without exception, contains granules which play a passive rôle in movement. The granules are albuminous, fatty, and in some cases inorganic (e.g. calcium carbonate in certain Myxoplasmodia) in nature. Often the exterior portions of the cell (exoplasm) are free from granules, while they are present in large quantities in the internal regions (endoplasm). The irregular shaking, dancing motion of these particles, called the 'Brownian movement,' must not be mistaken for vital movements.

In addition to granules, protoplasm in vegetable cells always, in animal cells often, exhibits vacuoles or spaces filled with a watery liquid. These are globular in resting protoplasm, but may become drawn out during movement. The same holds good for gas bubbles,[2] which occur occasionally in protoplasm.

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[1] *Quart. J. Mic. Science*, xxiv. 373.

[2] Engelmann, *Pflüger's Archiv*, ii. 307.

Protoplasm is generally weakly alkaline (probably due to alkaline phosphates) or neutral ; in *Æthalium septicum* it is always distinctly alkaline. After death or after prolonged activity, cells and organs containing large quantities of cells become acid ; the acid produced is not merely carbonic acid, but as a rule lactic acid in addition.

Protoplasm contains 80–85 per cent. of water ; 15–20 per cent. of solids.

The solids present are chiefly proteids, but in addition small quantities of fat, carbohydrates like glycogen and inosite, and inorganic salts, especially of potassium, are present. Lecithin is also frequently present, and often ferments or enzymes can be separated from the cells.

The foregoing is a brief *résumé* of the known facts concerning the structure of protoplasm ; recent researches both in animal and vegetable histology have, however, amplified our knowledge in certain particulars, and certain new terms have been introduced to express certain new and definite facts. We will therefore next take up the points that require to be more fully dealt with.

The structure of Protoplasm : is it homogeneous?—According to the observations of Heitzmann, Froman, Klein, Carnoy, and others, the cell-protoplasm (or *cytoplasm* as it is often called, to distinguish it from the *nucleo-plasm* or substance of the nucleus) is composed of a fine network, *reticulum*, or spongework of fibrils, with a more fluid material in its interstices. These observers consider that the granules observed in protoplasm are chiefly the optical sections or crossing points of the fibrils ; at the same time true granules or *microsomes* may occur in the interstitial fluid.

Other histologists (Schäfer, Rabl, &c.), while not denying that such a reticulum may sometimes occur, yet consider that it is, as in the Amœba, often absent. The protoplasm of cells is often highly vacuolated ; it often contains rods (as in cartilage cells), or has a striated appearance (as in cells of the ducts of the salivary glands). In other cases again the appearance of a reticulum appears only after treatment with certain reagents, and may be a kind of coagulation produced by those reagents.

Carnoy believes that the reticulum consists largely of a substance called *plastin*, which we shall describe more fully in connection with the nucleus, where it is also found.

The following terms for these two different portions of protoplasm are used by different observers :—

The reticulum is called :

Protoplasma by Kupffer[1] (including also granules).

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[1] *Sitzungsb. d. math.-phys. Klasse d. k. Bayr. Akad. München*, 1882, vol. vi. *Bayr. Fischerei-Zeitung*, 1886.

Cytohyaloplasma by Strasburger.
Substantia opaca by Leydig.
Mitom by Flemming.

The inter-reticular, more fluid substance is called :

Paraplasma by Kupffer.
Cytochylema by Strasburger.
Substantia hyalina by Leydig.
Paramitom by Flemming.

Strasburger further distinguishes in the cytochylema :

Plasmochyma, the portions rich in proteids.
Cytochyma, the more watery sap in the vacuoles.

Schwartz,[1] who has examined protoplasm and nuclei microchemically, describes the following constituents in cell protoplasm or cytoplasm.

1. Plastin, a sticky, thready mass which resists peptic and tryptic digestion ; and is insoluble in concentrated potassium hydrate and sodium chloride solutions (1 in 10). He further distinguishes between cytoplastin (the plastin of cytoplasm), and chloroplastin (a similar substance found in chlorophyll grains).

2. Microsomes, the granules of the protoplasm ; insoluble in water. These may be absent.

3. The materials dissolved in the vacuoles, which in plant cells are always present. This fluid is stated to be sometimes acid, sometimes alkaline. The contents of vacuoles will be discussed more fully later on.

Few histologists deny that protoplasm often shows reticulum and enchylema, to adopt Carnoy's terms. This view of the structure of cells is specially interesting to the physiologist, as in the cells which become muscular fibres, the reticulum takes on a definite arrangement, different from the irregular disposition it has in primitive cells. This orderliness of arrangement is accompanied by the ordered and specialised movement known as muscular contraction.

It is, however, necessary to guard against the assumption that the reticulum is firm, for it is only slightly less fluid than the enchylema.

The Proteids of Protoplasm.—The largest chemical constituent and the only constant one in protoplasm is proteid matter. Various theories to account for the differences between living and non-living proteids have been already dealt with (p. 115).

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[1] *Die morphol. u. chem. Zusammensetzung der Protop.* Breslau, 1887.

In the case of the lymph cells occurring in lymphatic glands, and of the liver cells, large masses of cells, very little altered in structure from primitive cells, can be obtained; here the methods of macro-chemistry are applicable. These and other cases will be more fully considered in connection with those tissues, but for the present it may be here stated that as a general rule the proteids of cytoplasm are:

(1) Nucleo-albumins; compounds of a phosphorised substance with a proteid. This substance is probably identical with the substance called plastin by those who have employed the methods of micro-chemistry.

(2) Globulins: one in small amount with a heat-coagulation temperature of about 50°C.; another (cell globulin) in larger amount, which resembles serum-globulin in its heat-coagulation temperature (75°C.).

(3) An albumin in small quantities resembling serum-albumin in its characters; this, however, is often absent.

(4) Albumoses and peptones in small quantity, and generally produced as a result of *post-mortem* changes, or of retrogressive changes occurring within the body, as in the degenerative changes that occur in pus cells.

The contents of Vacuoles.—A vacuole is a globular cavity containing a watery fluid. Vacuoles appear to be always present in vegetable cells; in animal cells they also frequently occur.

In unicellular organisms like the amœba, solid particles when ingested are surrounded with liquid; this liquid may be taken in with the solid, but generally it is poured out by the cell around the solid, and appears to play the rôle of a digestant. Other vacuoles, such as the contractile vacuole of the amœba, are excretory.

Engelmann found that blue litmus grains when ingested by amœbæ and other protozoa became red after a time. A. G. Bourne[1] suggests that this may be due to an acid secreted in an attempt to digest the particles. If vorticellæ take in aniline blue, the protoplasm becomes filled with blue vacuoles, the contents of which are gradually absorbed, the pigment reappearing of a different tint in the contractile (excretory) vacuole. Schäfer and Eckstein[2] found that blue litmus granules taken in by white blood corpuscles remain unchanged in colour.

Miss Greenwood[3] has made a very exhaustive study of the processes of digestion in Rhizopods, especially in amœba and actinosphærium, with the following results:—

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[1] *Quart. Journ. Microsc. Science*, xxiv. 377, foot-note.

[2] *Quain's Anat.* vol. ii. p. 5.

[3] *Journ. of Physiol.* vii. 253; viii. 263.

(1) The ingestion of solid matter is promiscuous in amœba; that is, nutritious and innutritious particles are taken in with equal readiness. Actinosphærium, on the other hand, rarely ingests innutritious particles.

(2) The nutritious particles are in both animals digested by fluid poured out around them. This fluid has no action on the cuticle of organisms, on cellulose, on siliceous cell-walls, on fat, or on starch. It is colourless; it digests proteid matter, especially uncoagulated proteid. It changes chlorophyll to a dark-brown colour (especially in amœba). It has no action on litmus or carmine particles accidentally inclosed with nutritious matter, and is therefore neutral in reaction. Innutritious matter does not become surrounded by this fluid.

(3) The secretion is more active in actinosphærium than in amœba.

(4) Ejection is performed at the hinder end of the amœba, either by means of a vacuole, or often (for instance, when algæ are taken in) without one. In actinosphærium an excretory vacuole is always present.

(5) The time between ingestion and ejection is difficult to determine, and varies with the size and digestibility of the ingesta from 3–4 days in amœba, from 1½ to 8 hours in actinosphærium.

THE NUCLEUS

A large amount of original work has during the last twenty years been carried out, bearing on the structure and changes that occur in the nucleus of cells.

The existence of the nucleus was discovered by Robert Brown in vegetable cells in the year 1821, and in animal cells by Th. Schwann nearly twenty years later. It was at first described as a more solid structure in the interior of the protoplasm or vitellus of the cell, containing in its interior, one or several still more solid particles called nucleoli. We now know that the nucleus consists of a spongework of fibrils, permeating which is a more liquid substance, the nuclear matrix. The particles called nucleoli may be thickened portions of these fibrils, or they may float free in the nuclear matrix. This is universally true for all nuclei, but these structures vary in size and shape in different cells of the body. Spontaneous changes in form may occur in nuclei liberated by the rupture of cells (Stricker, Flemming, Klein), which have been compared to the amœboid movements of protoplasm.

Histologists distinguish between the 'resting' or non-dividing nucleus and the 'dividing' nucleus. When cells divide, the nucleus first undergoes certain well-marked and definite changes; the fibrillar material is arranged into definite patterns (skeins, stars, rosettes, &c.); these separate into two groups which form the foundation of the daughter nuclei. The name given to this series of changes is *karyokinesis* or *karyomitosis*. The division of the cell protoplasm follows that of the nucleus.

Until comparatively recently, the chemical structure of the nucleus was unknown; it was spoken of vaguely as being composed of 'germinal matter'; but now, thanks to the labours of Miescher, Zacharias, Kossel, and others, we know of certain definite chemical substances in the nucleus, such as nuclein, plastin, and adenin. Other substances have also been described, but as their existence rests chiefly on microchemical reactions, one must be cautious at present in regarding them as distinct chemical units.

The Resting Nucleus

The resting nucleus consists (*see* fig. 45) of :—

(1) An outer investing membrane ; the nuclear membrane.

(2) A network of fibrils throughout its substance ; the nuclear network.

(3) Nucleoli.

(4) The more liquid material in the meshes of the network ; the nuclear sap or nuclear matrix.

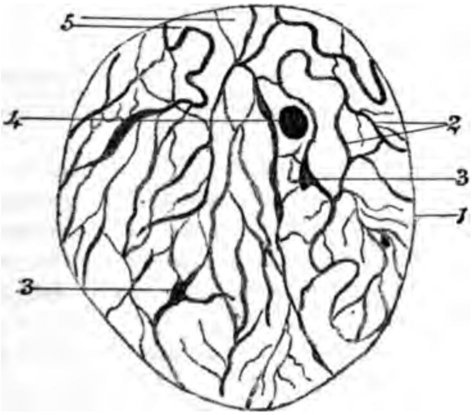

FIG. 45.—Diagram of resting nucleus. 1. Nuclear membrane. 2. Nuclear network. 3. Net-knots. 4. Nucleolus. 5. Nuclear matrix. (After Waldeyer.)

These different parts must be taken one by one :—

The nuclear network.—This consists of fibres thick in parts, thin in other parts, arranged according to most observers irregularly. Balbiani describes this network in the chironomus larva as being composed of a single, much twisted thread. Flemming has, however, shown that this is by no means a widespread occurrence; and some regard it still doubtful whether the numerous threads which are present, do or do not anastomose.

Rabl[1] considers that even in the resting nucleus, the fibres have a regular arrangement; he distinguishes between primary and secondary fibres; the primary are the thicker ones, which run from one aspect of the nucleus, called the *polar field*, where they loop round and part from one another; they spread over, and throughout the nucleus in a radiating way, and at the opposite end of the nucleus, or *antipolar field*, they are free and show no special arrangement. They are depicted in the left-hand half of fig. 46. The secondary fibres are finer, and by connecting the primary fibres form the network, as we have already described it, and thus render it very difficult to identify the primary fibres in the resting nucleus.

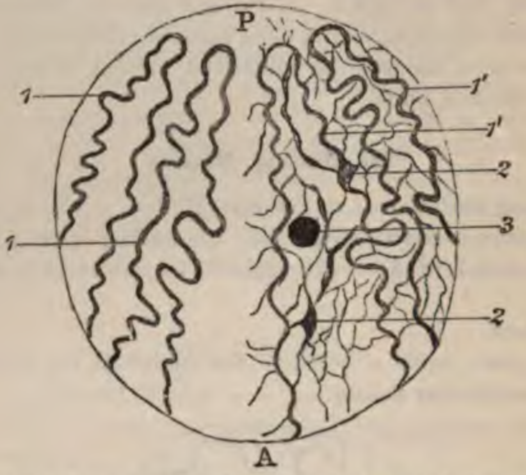

FIG. 46.—Scheme of resting nucleus (after Rabl). P, Polar area. A, Antipolar area. The left-hand part of the figure shows primary fibres (1, 1) only; the right-hand part of the figure shows the primary fibres (1′, 1′) connected into an anastomosing network by secondary fibres. 2, 2, Net-knots; 3, Nucleolus.

Dilute acids (acetic, formic, &c.) render the whole nucleus more apparent; water causes it to swell. Nearly all staining reagents (acid carmine, logwood, safranin, &c.) colour the network and nucleoli very intensely, while the interfibrillar substance remains uncoloured, or is only coloured faintly. From this difference in behaviour to stains, Flemming distinguishes in the nucleus between *chromatic* and *achromatic* substances.

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[1] *Morph. Jahrb.* x. (1885), p. 214.

The chromatic substance, or chromatin, includes the network and the nucleoli; and is, as E. Zacharias[1] has shown, identical with nuclein.

The achromatic substance is the interfibrillar material; during karyokinesis, part of this becomes arranged into a spindle-shaped collection of fibres.

Pfitzner[2] uses these terms more fully as follows:—

Chromatin = substance of the nuclear network.
Prochromatin (later, pseudo-chromatin) = nucleoli.
Achromatin = nuclear matrix.
Parachromatin = spindle figure.

Schwartz[3] uses the following terms:—

Chromatin = the nuclear network.
Linin = the spindle figure.
Paralinin = Flemming's achromatin (probably a globulin).
Pyrenin = nucleoli.
Amphipyrenin = nuclear membrane.

These names are given, not from the chemical properties, but chiefly from the microscopic appearances of the structures in question.

A very important step in our knowledge of the chromatic fibres was the discovery of Balbiani and Pfitzner, that they are made up of a number of granules or discs (their form is still uncertain) regularly arranged in single or multiple rows. Carnoy[4] believes that the filaments though chiefly composed of nuclein have an outer shell of plastin, an observation confirmed by van Bambeke.[5]

The nucleoli present many difficulties; the chief doubtful point concerning them is their relationship to the network. Flemming and Pfitzner regard them as different from the network, and not connected to it; others (Klein[6]) consider them as merely thickened portions of the network, and composed of the same material. No doubt such nucleoli (net-knots in figs. 45 and 46) do occur. But in addition there appear to be true nucleoli in Flemming's sense—rounded bodies free in the meshes of the network, floating in the nuclear matrix, and behaving differently to reagents.

Zacharias states that they consist of a shell of plastin, and their interior of proteid matter; they are apparently not composed of nuclein

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[1] *Botan. Zeitung*, 1881, 1882, 1885, 1887.

[2] *Morph. Jahrb.* vii. 1881, p. 289

[3] *Die morphol. u. chem. Zusammensetz. d. Protop.* Breslau, 1887.

[4] *La Cellule*, vols. i. and ii.

[5] *Arch. de Biol*, viii. 1887, p. 349.

[6] *Quart. J. Mic. Science*, xviii. July 1878; xix. p. 125.

Ogata,[1] Lukjanow,[2] and Stolnikow[3] distinguish nucleoli into karyosomes, plasmosomes,[4] and hyalosomes, according to their behaviour to eosine, nigrosine, safranine, and other stains. During cell division the nuclei dissolve in the nuclear matrix.

The nuclear matrix is not a simple watery fluid, but is rich in proteids. Various reagents cause the appearance in it of a fine precipitate, which one must guard against looking upon as a structure. Carnoy has apparently fallen into this error, when he describes a fine network of plastin in the nuclear matrix.

The nuclear membrane is regarded by some as the optical appearance presented by the termination of the nuclear network; but most observers agree that a true membrane is present. This is achromatic (Flemming, Strasburger, Pfitzner); it is formed from the cell protoplasm (cytoplasm) which lies next the nucleus, and is sometimes termed the inner cell membrane.

The Dividing Nucleus

It has been known since 1824 (Prévost and Dumas) that cells multiply by the subdivision of existing cells. v. Mohl, Remak, and Virchow showed that spontaneous generation of cells does not occur, the latter summing up the situation in the now classical phrase, 'omnis cellula a cellula.'[5] Among the earliest who watched the complete process of cell division in amœbæ and white blood corpuscles under the microscope were Stricker, Klein, Schulze, Ranvier, and Waldeyer. Remak's scheme of division was very simple: he stated that first the nucleolus, then the nucleus, and lastly the cell split into two parts, and so formed two daughter cells.

Direct nuclear subdivision (*à la* Remak) is called by Flemming *amitotic*, as distinguished from the *mitotic* or *karyomitotic* subdivision of the same author. In the latter there is a highly characteristic arrangement of fibres in the form of figures, which replace the nucleus; this separates into two parts, and from each of these parts a daughter nucleus is formed, and the division of the cell protoplasm follows. The more carefully the cases of so-called direct cell division are examined the fewer do they appear to be. There are certainly variations in different cases, and, as Carnoy says, no stage seems to be absolutely essential: some nuclei, for instance, are very poor in chromatin; and the differences that do exist are only of degree, not of kind.

The chief characteristics of a dividing nucleus (the chromatic nuclear figure, the achromatic spindle, and the cytasters) were first described by A. Schneider,[1] and subsequently rediscovered by Butschli[2] and Fol.[3] The name *karyokinesis* was applied to the series of changes by Schleicher.[4] Since then our knowledge of the process has been furthered by numerous investigators, but especially by Strasburger,[5] Flemming,[6] E. v. Beneden,[7] and more recently by Rabl.[8]

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[1] *Arch. f. Physiol. u. Anat. Physiol. Abth.* 1883.

[2] *Ibid.* 1887, p. 66.

[3] *Ibid.* 1887, p. 1.

[4] The plasmosomes are stated to wander out from the nuclei in certain cases to form para-nuclei (Lukjanow).

[5] *Arch. f. pathol. Anat.* viii. 23 (1855).

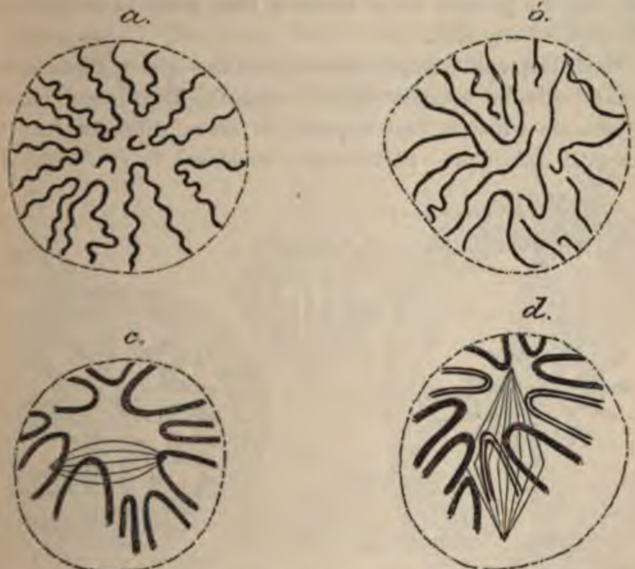

Fig. 47.—*Karyokinesis.* The skein or spirem stage. *a*, View of the nucleus from the polar area; *b*, from the antipolar area (dense skein); *c*, later stage (loose skein); the chromatic loops are thicker, shorter, and less twisted; the spindle makes its appearance; *d*, end of the skein stage. The chromatic loops are splitting longitudinally into sister threads; the spindle has taken up the position in which it will remain till cell division is over. (After Waldeyer.)

A full account of karyokinesis is obviously out of place in this work, and therefore the following brief account (after Waldeyer)[9] must suffice.

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[1] 'Unters. ü. Plathelminthen,' *Jahrbuch der Oberhessischen Ges. für Natur. und Heilkunde*, 1873.

[2] *Zeit. f. wiss. Zool.* 1875, xxv. 201.

[3] *Arch. de Zool.* iv. (1875).

[4] *Centralbl. med. Wiss.* 1878, p. 418. Carnoy's term is Cytodieresis.

[5] Strasburger, *Zellbildung u. Zelltheilung*, 3rd edit. 1880. *Kern und Zelltheilung*, 1888, Jena (see *Nature*, Nov. 1, 1888). *Arch. mikr. Anat.* xxi. 476; xxiii. 246.

[6] Flemming, *Zell-Substanz, Kern u. Zelltheilung*, Leipzig, 1882. *Arch. mikr. Anat.* xx. 1; xxiii. 141; xxiv. 50 and 338; xxix. 389. *Biol. Centralbl.* iii. 641. *Arch. f. Anat. u. Physiol. Anat. Abth.* 1885, 223. *Zool. Anzeiger*, 1886, no. 216.

[7] E. van Beneden, *Bull. acad. roy. de Belgique*, 1870, 1874, 1875, 1876, 1884, 1887.

[8] *Morph. Jahrb.* x. 214.

[9] An excellent summary of the present state of our knowledge on this important question will be found in Waldeyer's paper 'Ueber Karyokinesis,' *Arch. mikr. Anat.* xxxii. This paper has been translated by Dr. Benham (*Quart. J. Micros. Science*, xxx 159, 215).

The process may be divided into the following stages :—

1. The resting nucleus.

2. The skein or spirem stage ; the nucleoli dissolve, and the nuclear matrix then becomes more stainable ; the secondary fibres (Rabl) disappear, and the primary loops running from polar to antipolar regions remain (figs. 47 *a* and *b*).

3. Each loop splits longitudinally into two sister threads, and the achromatic spindle[1] appears (fig. 47 *c* and *d*). The direction of the axis of the spindle when it first appears is often different from that it subsequently takes up. Strasburger considers it is formed of cytoplasm which has intruded into the nucleus (in some cases through pores in the nuclear membrane). Carnoy thinks the dissolved nucleoli contribute to its formation ; Flemming, that it is formed from the achromatic substance ; Platner, that it may have a double origin, i.e. from both achromatic substance and cytoplasm.

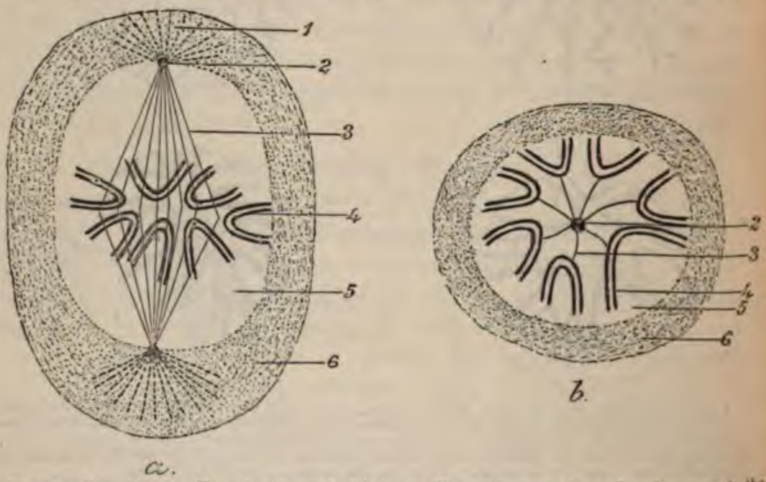

FIG. 48.—*Karyokinesis.* Monaster or equatorial stage. The nuclear membrane has disappeared ; the protoplasm of the cell is divided into a clear inner (5) and granular outer zone (6). The spindle (3) terminates at each pole in the polar or central corpuscle (2) round which the granules of the protoplasm are radially arranged to form a cytaster (1). The chromatic fibres (4) now each longitudinally split into two sister threads are grouped around the equator of the spindle. *a* is a view from the side ; *b*, from one pole of the nucleus. (After Waldeyer.)

4. The equatorial stage ; monaster. The nucleus has now two poles—those of the spindle. The spindle terminates in two polar corpuscles.[2] The nuclear membrane is lost, and thus cytoplasm and

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[1] The spindle or karyaster is better marked in vegetable than in animal cells, it is occasionally cylindrical in shape, it is but little affected by stains, it is rendered more apparent by dilute acids, but readily dissolves in artificial gastric juice.

[2] These are composed of nuclein and are partly derived from cytoplasm (Carnoy). They are absent in plant cells.

nuclear matrix become continuous; the cytoplasm separates into a clear and a granular zone, and the granules arrange themselves radially from the polar corpuscles (cytasters).[1] The chromatic fibres sink to the equator of the spindle, and arrange themselves so as to project horizontally from it (*see* fig. 48 *a* and *b*).

5. Metakinesis. The sister threads separate, one going towards one pole, the other to the other pole of the spindle (fig. 49); one set of sister threads form one daughter nucleus, the other the other.

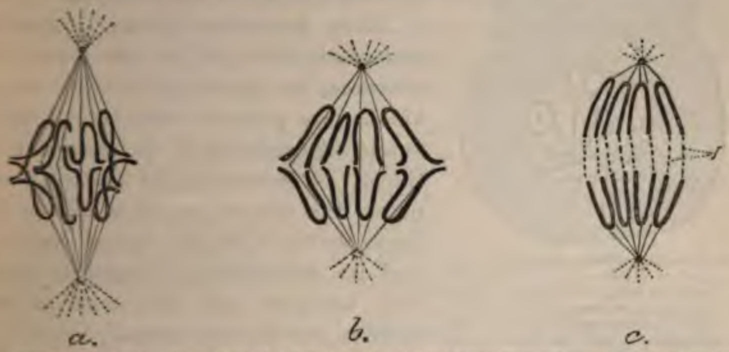

FIG. 49.—*Karyokinesis.* Separation of sister threads; (Metakinesis) one set moving toward one pole of the spindle, the other towards the other. *a*, *b*, and *c*, show successive steps in the process. In *c* (*f*) the uniting filaments (v. Beneden) are seen; and the appearance from each pole is like that in fig. 48 *b*, except that the chromatic fibres are single not double. This stage (*c*) is called the Dyaster or daughter star stage.

6. Dyaster, or daughter star stage; this stage occurs when the two sets of sister threads are separated, as in fig. 49 *c*. The fibrils which still unite them are regarded by v. Beneden as different from the spindle, which gradually disappears; Strasburger believes these are the spindle fibres along which the chromatic filaments shift. Each daughter nucleus then goes backwards through the same series of changes; the dyaster is followed by the

7. Dispirem or daughter skein stage (upper part of fig. 50). The new nuclear membrane begins to form in this stage at the antipolar region, and the polar corpuscle disappears. The cell itself then divides; the cell membrane being formed in plants by thickenings or knots in the equatorial region of each spindle fibre; these thickenings coalesce. They are called dermatosomes, and are absent in animal cells.

8. The resting daughter nuclei; when the cytoplasm has divided, the remains of the spindle disappears, the chromatic fibres become more twisted, lose their equal calibre, and become connected by secondary fibres, as is shown in the lower nucleus (figure 50).

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[1] Also called aureola and helioma.

In the egg cells of certain animals when dividing (e.g. Ascaris megalocephalus, v. Beneden), the chromatin filaments are but little marked; the whole or nearly the whole of the granules of the cell protoplasm are arranged in a radial way round the extremities of the spindle. At each end of the spindle is a polar corpuscle, and a spherical mass of protoplasm which acts as an *attraction sphere* surrounds it.

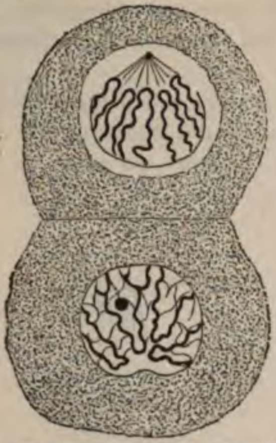

FIG. 50.—*Karyokinesis*, final stages. The place of division of the cell protoplasm is seen. The upper nucleus still shows the remains of the spindle. The chromatic loops are now twisted (daughter skein). The lower nucleus is further advanced; the position of the spindle is marked by a depression or hilus, the polar area of the new nucleus. The primary loops have become connected by secondary fibres, and a nucleolus has appeared (resting daughter nucleus).

Each attraction sphere consists of protoplasm arranged in two zones. The cytasters, as generally seen in karyokinesis, are probably due to a less highly developed condition of the same state of things. v. Beneden calls the polar corpuscle the central corpuscle, and he believes that it is in the central corpuscle and its surrounding attraction sphere that we must seek the cause of subdivision, not in the nucleus.

We must next proceed to examine the properties of the various chemical substances found in the nucleus, many of which have been alluded to in the foregoing account of its structure.

Nuclein

Dr. Lauder Brunton[1] was the first to investigate the chemical composition of cell-nuclei. He separated the nuclei from the red corpuscles of birds, by shaking them with a mixture of ether and water; the undissolved nuclei floated at the junction of the two liquids. Brunton described the nuclei as consisting of a mucin-like substance. Plósz[2], however, found on analysis that it was not mucin, as it contained a high percentage of phosphorus, an element absent from mucin. He considered it to be identical with the substance separated by Miescher[3] from pus corpuscles, and termed by him nuclein. The method adopted by Miescher was to subject the pus to gastric digestion; the nuclein alone remained undissolved.

Later Miescher[4] prepared a similar substance from the spermatozoa of different animals and from the yolk of hens' eggs, Hoppe-Seyler,[1] Kossel,[2] and Loew[3] from yeast, Plósz[4] from the liver, von Jaksch[5] and Geoghegan[6] from the brain, Lubavin[7] from cow's milk, and Worm-Müller[8] from yolk of egg. In fact, wherever nuclei are present, a substance is found which is rich in phosphorus, soluble in weak alkalis, insoluble in weak acids and in artificial gastric juice, and with the sticky character of mucin to a certain extent. A similar body is also found in the substances, like milk and yolk of egg, which form the food of the young animal. This substance is termed nuclein.

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[1] *Journ. of Anat. and Physiol.* 2nd series, iii. 91.

[2] *Hoppe-Seyler's Med. Chem. Untersuchungen*, Heft iv. (1871), 460.

[3] *Ibid.* p. 441.

[4] *Verhandl. der nat. Ges. Basel*, vi. (1874), Heft i.

Nuclein is a compound of carbon, hydrogen, nitrogen, sulphur, phosphorus, and oxygen. Elementary analyses of nuclein from different sources yield very discordant results. The following examples may be quoted :—

	From Pus (Hoppe-Seyler)	From Spermatozoa of Salmon (Miescher)	From Human Brain (v. Jaksch).
C	49·58	36·11	50·6
H	7·10	5·15	7·6
N	15·02	13·09	13·18
P	2·28	9·59	1·89

The nuclein from spermatozoa differs from other nucleins in containing no sulphur. Miescher's formula for it is $C_{29}H_{49}N_9P_3O_{22}$. From these results we must conclude, either that nuclein is not a chemical unit, but a mixture of organic phosphorus compounds with proteids or proteid-like substances (Worm-Müller), or more probably that several varieties of nuclein exist (Hoppe-Seyler).[9] Miescher himself found that some nucleins were more insoluble in alkalis than others. Kossel[10] confirms Hoppe-Seyler's view of the case, for he finds that on heating yolk-nuclein and milk-nuclein with weak acids, no bases rich in nitrogen like guanine and hypoxanthine are formed, whereas such bases are obtainable from cell-nuclei. Yolk-nuclein and egg-nuclein contain iron, cell-nuclei do not. A compound of nuclein with iron, called hepatin, is also found in the liver (Zaleski).[11]

An intermediate product between nuclein and hypoxanthine is called *adenine* ($C_5H_5N_5 + 3H_2O$) by Kossel,[12] its discoverer. It crystallises in the rhombic system, forms compounds with bases, acids, and salts. On heating it with sulphuric acid, NH is replaced by O, and hypoxanthine is thus formed ($C_5H_5N_5 + H_2O = C_5H_4N_4O + NH_3$). Both adenine and hypoxanthine contain a radicle $C_5H_4N_4$ called adenyl. (*See* also p. 90.)

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[1] *Med. Chem. Unters.* iv. 500.

[2] *Zeit. physiol. Chem.* iii. iv.

[3] *Pflüger's Archiv*, vol. xxii. (1880).

[4] *Ibid.* vii.

[5] *Ibid.* xiii.

[6] *Zeit. physiol. Chem.* i.

[7] *Ber. d. deutsch. chem. Gesellsch.* x. 2237.

[8] *Pflüger's Archiv*, viii. 1874.

[9] *Physiol. Chemie*, p. 85.

[10] *Zeit. physiol. Chem.* x. 248.

[11] *Ibid.* x. 453.

[12] *Ber. d. deutsch. chem. Gesell.* xx. 3356. *Zeit. physiol. Chem.* xiii. 395, 432.

Adenine is obtainable from both animal and vegetable tissues which are rich in cells ; it cannot be obtained, or only in small quantities, from muscle. It appears that muscular fibres which have lost the morphological characteristics of cells to a great extent, have also lost some of the chemical distinctions of cells ; but in those tissues the cells of which retain their original character, hypoxanthine and xanthine occur, not uncombined, but in union with other groups of atoms, especially with phosphoric acid and proteids as part of a still more complex union, nuclein. The word differentiation can thus be applied not only in a morphological, but also in a chemical sense to cells.

Artificial preparation of nuclein.—Liebermann[1] believes that nuclein is a compound of albumin with metaphosphoric acid. He finds that the composition and reactions of the precipitate obtained by adding this acid to a solution of albumin cannot be distinguished from those of nuclein. Pohl,[2] however, has shown that although this substance resembles nuclein in its solubilities, it differs from true nuclein (i.e. the nuclein from nuclei) in the fact that substances of the uric acid group (xanthine and hypoxanthine) are not obtainable from it on decomposition.

Plastin

This substance we have seen is described as forming an outer shell to the nucleoli, and to the chromatic filaments ; it is also present in the cytoplasm. Its existence rests to a large extent on micro-chemical evidence. It is very like nuclein, but is more insoluble. The fact that it is a different substance from nuclein supports the statement that has been already made as to the presence of several organic phosphorised compounds in the nucleus.

E. Zacharias[3] thus describes the micro-chemical differences between nuclein and plastin. After artificial gastric digestion two substances in the cell remain undigested ; one confined to the nucleus is characteristically bright and sharply defined. It has a special affinity for certain pigments, and is in fact chromatin or nuclein. The other substance, occurring both in the nucleus and the cytoplasm, and also in yolk-spheres, appears after treatment with gastric juice ill-defined and swollen. Though swollen by acids and other reagents (solutions of sodium chloride, sodium hydrate, &c.), it is very insoluble. It is dissolved by concentrated hydrochloric acid; it is also much more insoluble in alkalis than nuclein, and is stated to withstand pancreatic digestion.

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[1] *Berichte d. deutsch. chem. Ges.* xxi. 598.

[2] *Zeit. physiol. Chem.* xiii. 292.

[3] *Botan. Zeitung*, 1887, p. 281.

The term plastin was first applied to this substance by Reinke and Rodewald[1]; it appears to be identical with Miescher's insoluble nuclein. Like nuclein, it contains phosphorus (Reinke).[2] Löw[3] by treating it with alkalis has separated a proteid from it. These facts all correspond with the hypothesis that plastin is a nucleo-albumin; and there appear to be varieties of plastin, just as there are varieties of nuclein. Schwartz speaks of that in the cell protoplasm as cytoplastin, and that in chlorophyll grains as chloroplastin. He, however, uses the term plastin in a different sense from Zacharias, namely, as a name for the whole of the proteid matter of the protoplasm, and not for any special constituent of it. He regards the reticulum seen in the cell as an appearance due to the action of reagents, in fact, a kind of coagulation; in this opinion he differs from Carnoy (*see* p. 191).

Schwartz describes the plasmatic substratum of the chlorophyll grains as consisting of two proteids; one the chloroplastin mentioned above, which is not digestible by pepsin nor by trypsin; the other he calls metaxin. This is easily digested by both these ferments, and dissolves after swelling in very weak hydrochloric acid (1:1000).

Histon.—This was a proteid prepared from nuclei by Kossel[4]; it belongs to the group of proteids known as albumoses or propeptones. As Kossel extracted it from the nuclei by means of dilute hydrochloric acid, there is, however, but little doubt that it is an artificial product produced from the native proteids of the nucleus by means of the reagent employed.

FUNCTIONS OF CELLS

To the anatomist the single egg cell, or the unicellular organism, is an extremely simple object. To the physiologist on the other hand simplicity of structure means an increased difficulty in understanding function. In the higher animals certain cells are set apart specially to perform one function, certain other cells to perform another; some for instance are concerned in muscular contraction, others in elaborating secretions, others in reproduction, and so forth. But in such an animal as the amœba all these functions—movement, secretion, digestion, excretion, and multiplication—are performed by one cell. In the higher animals the various functions are unravelled from one another, but in the amœba, looking at its apparently simple structure, it is difficult to realise the potentialities of and the variety of functions inextricably blended in the little mass of living jelly.

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[1] *Unters. aus d. botan. Lab. Univ. Göttingen*, 1881.

[2] *Ibid.* 1883.

[3] *Bot. Zeit.* 1884.

[4] *Zeit. physiol. Chem.* viii. 511.

In the fertilised ovum of one of the higher animals we have a no less wonderful potentiality; though of so simple a structure, and so minute in volume, it possesses not only powers of multiplication, but also powers of directing the arrangement and subsequent changes in the cells so produced to form the complicated organs of the adult. In addition to this, the offspring resembles the parent in appearance, and often in subtler qualities, such as instinct, mental disposition, and even in tendencies to certain diseases like gout, syphilis, &c.; all these potentialities must have been present in the original ovum from which the rest of the body was formed.

While a cell is alive, it is always undergoing certain chemical changes. During assimilation it is building up its own substance from other material, which is called food. On the other hand it is undergoing retrogressive metamorphosis, and this is especially increased during activity. The destructive chemical changes in a muscle are for instance more marked during its contraction than when it is not contracting. The chief destructive changes that occur are of the nature of oxidation. Carbon unites with oxygen, and carbonic acid is given off; hydrogen unites with oxygen to form water; nitrogen is burnt off in the form of imperfectly oxidised substances, of which the chief are urea (CON_2H_4) and uric acid ($C_5H_4N_4O_3$); but other substances like xanthine, hypoxanthine, creatine, &c., are also formed, and will be generally found in minute quantities in organs composed of cells; sulphur passes off in the form of sulphates. These combustion changes represent a transformation of energy; the potential energy of chemical affinity is transformed, and exhibits itself partly as heat, partly as electrical change, partly in the form of mechanical work.

The series of changes beginning with assimilation and ending with excretion is what is known as *metabolism*; the cell is continually building up either its own substance, or materials like glycogen, ferments, fat, &c., within its substance[1]; then, on the other hand, it is continually undergoing a downward disintegrative process. Adopting Gaskell's nomenclature, constructive metabolism may be termed *anabolism*, destructive metabolism *katabolism*.

In the succeeding chapters we shall be dealing with the special functions of certain groups of cells; we shall then have to speak of the functions of the cells of the blood, of the liver, and of other organs; of uscular fibres (which are in origin cells), and of secreting cells. In he chapter on fermentation, the peculiar activity of those unicellular rganisms known as yeasts and bacteria has already been specially dealt with.

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[1] Substances formed within cells, glycogen, starch, fat, contents of vacuoles, &c., are often termed *cell-contents*.

FUNCTIONS OF THE NUCLEUS

The different views that are held with regard to the functions of the nucleus are for the most part hypothetical. The nucleus probably exercises in some way a directing or controlling influence on the cytoplasm, this being especially brought out by the part it plays during cell division; the nucleus divides first, the cytoplasm follows suit. The cytasters and radiating lines in the protoplasm around the poles of the spindle remind one forcibly of the effect produced by placing a magnet in the midst of some iron filings, the radiating position of the metallic fragments around the poles of the magnet indicating the direction of the lines of force. Though it is dangerous to carry such comparisons too far, the similar lines in the cytoplasm perhaps indicate the lines of the attractive force exerted by the poles of the spindle or of the closely allied attraction spheres of v. Beneden.

The nucleoli are believed to be collections of reserve material which enters into solution when karyokinesis begins, and perhaps contribute to the formation of either the achromatic or chromatic fibres. The nuclear matrix becomes more stainable after the nucleoli have entered into solution, and Strasburger attaches great importance to the stainable nuclear matrix, and believes that in vegetable cells it takes part in the formation of the new cell membrane.

Another function attributed to the nucleus is that of exercising a controlling influence on the nutritive or metabolic changes of the cell. By certain fluids a vegetable cell can be broken up within its cell wall into masses of protoplasm. Strasburger found that in *Funaria*, the chlorophyll corpuscles in the fragments of cells so obtained which were without a nucleus, were unable to form starch. Klebs found that in filaments of another plant *Spirogyra*, when broken up in this way (plasmolysed), a formation of starch goes on in masses of protoplasm destitute of a nucleus, but this is easily explained by supposing with Strasburger that the little bright bodies called pyrenoids physiologically replace the nucleus in this connection.

One of the most interesting of modern theories regarding heredity is that of Weismann, who speaks of the living substance transmitted from one generation to another as the germ-plasma. He further believes that the germ-plasma is situated in the nucleus of the reproductive cells.

COMPARISON OF ANIMAL WITH VEGETABLE CELLS

Both animal and vegetable cells are composed of nucleated masse of protoplasm, but exhibit points of difference in structure, of which th most striking is the presence of a cell wall in most vegetable cells[1] an its absence in most animal cells; the amount of vacuolation is als generally greater in vegetable cells. Both animal and vegetable protoplasm breathes oxygen and gives off products of oxidation, like carbonic acid. This process is, however, in the light counteracted in most vegetable cells by the activity of the green pigment chlorophyll.

Plants containing chlorophyll require simple chemical substances as food. Oxygen, traces of ammonia, and, under the influence of sunlight, carbonic acid are absorbed from the atmosphere. Water, ammonia and its salts, nitrates and other salts are taken up by the roots from the soil.

Carbon is obtained by the decomposition of carbonic acid; this is brought about in the light by the agency of the chlorophyll. Fungi and other plants devoid of chlorophyll obtain it by decomposing compounds in which carbon is combined with hydrogen.

Hydrogen is obtained from water.

Oxygen is obtained from the air.

Nitrogen from ammonia and its salts by lower plants, from nitrates by the higher plants.

Sulphur is obtained from sulphates.

Phosphorus is obtained from phosphates.

Potassium, magnesium, sodium, calcium, iron, &c., from various salts in the soil.

From these simple materials the plant builds up complex nitrogenous and non-nitrogenous bodies. Synthesis is in fact characteristic of all plants containing chlorophyll, but analytic processes also occur.

The formation of non-nitrogenous substances, such as starch, is directly connected with the action of chlorophyll under the stimulus of light. Kraus found that starch grains appeared in the chlorophyll corpuscles of *Spirogyra* within five minutes after exposure to bright sunlight, within two hours in diffuse daylight; in *Funaria* they were slower in appearing.[2]

It is probable that chlorophyll acts by causing the union of carbonic acid and water to make formic aldehyde, oxygen being eliminated, thus :—

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[1] Composed chiefly of cellulose. Paragalactin and other insoluble carbohydrates are also present (*see* p. 109).

[2] Vines, *Physiology of Plants*, p. 147.

$$CO_2 + H_2O = CH_2O + O_2$$

By polymerisation and dehydration the aldehyde becomes starch, thus :—

$$6CH_2O = C_6H_{12}O_6 \text{ (grape sugar)}$$
$$C_6H_{12}O_6 - H_2O = C_6H_{10}O_5 \text{ (starch)}$$

Cellulose, fats, non-nitrogenous acids, and nitrogenous substances like asparagin, leucine, proteids, &c., are built up probably by similar synthetical processes, but these have not yet been worked out.

The energy that enables the plant cell to do this work is undoubtedly the radiant energy of light, and more especially of those rays absorbed by the chlorophyll. When examined spectroscopically a solution of chlorophyll or a green leaf presents certain absorption bands (fig. 51). It is the light of just those parts of the spectrum that is most active in the decomposition of carbonic acid; the position of the maximum energy of light coincides with the maximum of absorption (Langley). Engelmann has shown that bacteria placed with a filament of *Cladophora* on the solar spectrum under the microscope, collect around the filament in the regions of the chlorophyll bands, that is, in the regions where most oxygen is being evolved.[1] A green plant kept in darkness soon dies, owing to its not being able to obtain carbonaceous food.

A somewhat elevated temperature is essential to the life of all plants. Light has apparently no influence on the true respiration (taking in oxygen and giving off carbonic acid) performed by the plant protoplasm.

In these synthetical processes the kinetic energy of the sun's rays is stored up and becomes potential.

Energy is, of course, expended during plant-life, but in less amount than it is stored. This stored energy is again liberated as heat in the combustion of fuel, or as heat and motion in the bodies of living animals.

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[1] More recently Engelmann (*Pflüger's Archiv*, xlii. 186) has demonstrated the same fact in another way. A spray of *Spirogyra* was mounted in a drop of diluted ox blood rendered venous by a stream of hydrogen. In one minute in direct sunlight, in fifteen minutes in diffuse daylight, the blood in the neighbourhood of the spray became arterial, and returned to its venous tint in the dark. If a spectrum was projected under the preparation, the change to the arterial tint occurred in the neighbourhood of those parts of the spray lying over the parts of the spectrum where the absorption bands of chlorophyll are situated, especially in the neighbourhood of the C line. There are other pigments which play a similar *rôle* to chlorophyll, i.e. decompose carbonic acid; one of these is bacterio-purpurin, a red pigment produced by the activity of certain bacteria (Ray Lankester) of the sulphur-bacteria class (Winogradsky, *Botan. Zeit.* 1887, no. 31–37). These pigments are all termed chromophylls; they act with regard to the spectrum *mutatis mutandis* similarly to chlorophyll.

The animal cell, on the other hand, receives its energy in only a small measure from the sun's heat, but the greater amount is received in the form of vegetable food, i.e. of the substances formed by synthetical processes during plant life. In the case of carnivorous animals, the supply of vegetable food is an indirect one through the body of another animal. The complex food stuffs either directly or indirectly enter into the composition of the cell protoplasm, and are there burnt off as simpler products (carbonic acid, water, urea, &c.). During this destructive metabolism, kinetic energy (heat, motion, electricity, &c.) is liberated. The animal cell stores a certain amount of potential energy, but this is less than that which is expended. In other words, the conditions of the green plant cell in sunlight are reversed. In green plant cells in the dark, and in plant cells without chlorophyll, the vital processes resemble those of animal cells. In those exceptional animals, the cells of which contain chlorophyll (e.g. *hydra viridis*), their behaviour in sunlight is like that of plant cells—they decompose carbonic acid, liberate oxygen, and store the carbon.

Speaking generally, the plant is chiefly concerned in synthesis, and so furnishes potential energy to the animal. The animal liberates this as kinetic energy, which, however, is not retransformed into potential energy for the plant. The plant receives new supplies of energy from the sun's rays.[1]

In a recent paper Pflüger[2] has pointed out that this contrast must not be pushed too far. The cell protoplasm itself acts in the same way in both animal and plant cells, breathing oxygen and giving out carbonic acid, water, and amido-compounds. Synthetic processes are undoubtedly more highly developed in plants containing chlorophyll, but they are present also in animal cells. The formation of hippuric acid from benzoic acid and glycocine, or of ethereal sulphates from phenol and sulphuric acid, are typical instances of syntheses occurring in animal cells. He also shows that syntheses must occur in the retrogressive metamorphoses that lead to the formation of uric acid and its congeners from proteids, in the formation of fat from proteid, or from carbohydrates, and in the formation of carbohydrates (glycogen) from proteids. These chemical operations performed by the living cell cannot be imitated in the laboratory or explained by any known chemical laws; there is no doubt, at first, an extensive breaking down of the complex molecules, and then the cells build up entirely new materials again, of a complex nature from the simple carbon compounds so liberated.

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[1] The foregoing account of the cell processes is very largely taken from McKendrick's *Physiology*, pp. 23–27.

[2] *Pflüger's Archiv*, xlii. 144.

The close resemblance between animal and vegetable cells is further shown by the fact that many lower plants (bacteria, moulds, &c.) not only flourish in solutions of albumin and sugar, but actually shed out ferments to convert proteid into peptone, and starch into sugar, to aid absorption. They breathe oxygen, produce carbonic acid, amido-derivatives, and, without the aid of sunlight, fat, carbohydrate, and proteid.

Nägeli[1] has shown that these fungi will assimilate carbon from compounds in which it is combined with hydrogen (amines, &c.), but not from those in which it is combined with nitrogen (cyanogen, &c.).

The question whether light has any influence in accelerating the chemical processes in animals, was answered in the affirmative by Moleschott[2] and v. Platen.[3] Speck[4] and Loeb[5] have, however, shown that light of itself does not cause the increased production of carbonic acid, but acts reflexly through the nervous system, especially through the retina, whereby increased muscular movements occur, and so an increase in the chemical processes takes place. Loeb took lepidopterous larvæ in the chrysalis stage when movements are absent, and found that oxidation processes were practically equal in those exposed to light and those kept in the dark.

APPENDIX. - CHLOROPHYLL[6]

The term chlorophyll was invented by Pelletier and Caventon;[7] it is the substance or mixture of substances to which the green colour of leaves and other vegetable organs is due.

It is an exceedingly unstable body, and most attempts to isolate it have failed, because in the processes adopted for the purpose decomposition has been brought about. Berzelius, Mulder, and Fremy employed strong mineral acids to extract it from leaves, under the mistaken impression that it was a stable body, but solutions of chlorophyll are destroyed by the action of air and sunlight, much more than by strong acids.

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[1] *Sitzungsb. Bair. Akad. Wiss.* 1879.

[2] *Wien. med. Wochensch.* 1885.

[3] *Pflüger's Archiv*, xi. 272.

[4] *Arch. f. exp. Path. u. Pharmak.* xii.

[5] *Pflüger's Archiv*, xlii. 393.

[6] The following account of the chemistry of chlorophyll is almost entirely an abstract of a paper by Dr. Schunck on that subject in the *Annals of Botany*, vol. iii. pp. 65–120.

[7] *Annales de chimie et de physique*, ix. 194.

Gautier[1] obtained a solution of chlorophyll by the use of neutral solvents, like alcohol and ether, and stated he obtained green crystals which he considered to be composed of the pure pigment, but Hansen[2] did not succeed in obtaining them. Hansen employed caustic soda as a solvent; this saponifies the fat which accompanies the chlorophyll. A yellow pigment is removed from the mixture by light petroleum, and then the chlorophyll is dissolved out by a mixture of alcohol and ether. On evaporating the solvent, dark green sphæro-crystals of 'chlorophyll-green' are left. These crystals can, however, hardly be composed of pure chlorophyll, as they are easily soluble in water, a medium in which chlorophyll is insoluble; and in a later communication Hansen himself has admitted that his crystals contain sodium.

In view of the difficulty found in isolating chlorophyll, our knowledge of its chemical and physical properties is necessarily limited. It is insoluble in water, and soluble in substances which, like alcohol, ether, carbon disulphide and chloroform, dissolve fats. These solutions show a green colour with a red fluorescence.

Spectroscopically a solution shows four distinct bands and two indistinct bands. The two latter, distinguished as bands V and VI, are situated as is seen in figure 51 at the blue end of the spectrum, and are only visible by sunlight in dilute solutions. Some observers consider that these are not true chlorophyll bands, but belong to a yellow colouring matter which accompanies chlorophyll, and which is called xanthophyll. Kraus[3] and Sachsse[4] have partially succeeded in separating the two pigments.

Elementary analyses of chlorophyll have yielded most discordant results; two of the latest determinations that have been made will serve to illustrate this statement.

	Gautier	Hansen
Carbon .	73·97 per cent.	67·26 per cent.
Hydrogen .	9·80 ,,	10·63 ,,
Nitrogen .	4·15 ,,	5·12 ,,
Ash . .	1·75 ,,	

Oxygen is also present; when burnt, chlorophyll leaves an ash which contains phosphates of calcium and magnesium and a little ferric oxide. The ash has an acid reaction due to acid phosphate. The phosphates may be derived from phosphorus in the chlorophyll, or in an impurity; there is equal doubt with regard to the iron, whether or not it is contained in the chlorophyll molecule. Most observers agree in regarding chlorophyll as a substance, the molecules of which are in a state of unstable equilibrium.

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[1] *Compt. rend.* lxxxix. 861.

[2] *Arbeiten d. Bot. Inst. Würzburg*, iii. 123 and 430.

[3] *Zur Kentniss d. Chlorophyllfarbstoffe*, Stuttgart, 1872.

[4] *Die Chemie u. Physiol. d. Farsbt.* Leipzig, 1877.

Decomposition products of chlorophyll.—Hoppe-Seyler[1] extracted fresh grass with boiling absolute alcohol; the extract on being allowed to stand, deposited crystals which were purified by recrystallisation: the substance so obtained, he termed *chlorophyllan.* It melts at 110°C. to a black liquid, which on further heating burns with a luminous flame. It is easily soluble in ether, light petroleum, benzol, and chloroform. Its solutions show the characteristic first band of chlorophyll, but the remaining bands differ from those seen in fresh plant extracts. Hence it is probably a decomposition product of chlorophyll. Its percentage composition is C, 73·34; H, 9·72; N, 5·68; P, 1·38; Mg, 0·34. On treatment with hot alcoholic potash, it yields a black crystalline acid (chlorophanic acid), glycero-phosphoric acid, and neurine. Hence chorophyllan is probably a lecithin.

By the combined action of ether and hydrochloric acid Fremy[2] obtained two pigments from chlorophyll, a yellow and a blue. The yellow pigment dissolved in the ethereal fluid, the blue one in the acid below it. The names *phylloxanthine* and *phyllocyanine* were respectively given to these colouring matters. Schunck confirms Fremy's results in the main, and gives a full account of the chemical, physical, and spectroscopic appearances of these two substances in the memoir already referred to. Phyllocyanine is in contrast to chlorophyll very stable; it is a weak base, and forms compounds with zinc, copper and other metals.

Phylloxanthine is more difficult to purify than phyllocyanine. It must be carefully distinguished from xanthophyll, to be described later.

Whether phylloxanthine is converted into phyllocyanine by the continued action of the acid, or whether the two pigments are formed independently, but in succession, from chlorophyll, or whether lastly the two owe their formation to two distinct substances which together constitute ordinary chlorophyll, must be still considered doubtful.

Alkalis cause a decomposition or change in the chlorophyll; Hansen's chlorophyll-green is a product of this kind, and Schunck has obtained a crystalline product he terms *phyllotaonin.* Alkali first converts chlorophyll into a substance of which chlorophyll-green is the sodium compound; on decomposition with acids this yields phyllotaonin, which in a nascent state in contact with alcohol and ether undergoes etherification. It has the following percentage composition : C, 66·49 ; H, 6·58 ; N, 3·32 ; O, 23·61. Schunck has further described some interesting experiments on the action of aniline on chlorophyll.

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[1] *Zeit. physiol. Chem.* iii. 889; iv. 198; v. 75.

[2] *Comptes rend.* l. 409; lx. 188; lxxxiv. 983. On the subject of the decomposition of chlorophyll by acids *see* also Filhol, *Ibid.* lxvi. 1218; lxxix. 612 (who describes a black crystalline substance), Russell and Lapraik, *Journ. Chem. Soc.* xli. 334 (this deals especially with spectroscopic appearances).

Substances accompanying chlorophyll.—Berzelius[1] supposed that the yellow colour of autumn leaves was formed from chlorophyll in consequence of changes induced by cold ; he termed it xanthophyll. Kraus endeavoured to show that ordinary chlorophyll is a mixture of two pigments—a bluish one, cyanophyll, and a yellowish one, xanthophyll ; and there is good reason to suppose that the xanthophyll of autumn leaves is merely the yellow pigment left after the fading of the blue. It is, however, doubtful if the yellow colouring matter of etiolate leaves, of green leaves, and of autumn leaves is the same. Stokes[2] separated two green and two yellow pigments. Tschirch calls the yellow pigment of etiolate leaves etiolin, and separates it widely from the xanthophylls, of which he describes five. Schunck has found the yellow pigment of faded leaves to consist of two distinct yellow colouring matters, differing in solubilities and in spectroscopic appearances. Of all these substances one only, *chrysophyll* (Hartsen, the erythrophyll of Bougarel), has been obtained in a pure state. Leaves are extracted with boiling alcohol ; the extract on standing deposits red crystals, mixed with fat and chlorophyll ; the deposit is dissolved in chloroform, filtered, and alcohol added to the filtrate, crystals again form on standing, and may be obtained pure by repeating the process several times. Solutions of this substance show two bands at the blue end of the spectrum, coinciding very nearly with bands V and VI of the ordinary chlorophyll spectrum. One other xanthophyll, at least, and probably the remainder give no spectroscopic bands.

It is perhaps one of the xanthophylls to which is due the glucose reaction observed by Schunck[3] after treating chlorophyll solutions with acids, since the substance is to a great extent removed by agitating the solutions with carbon disulphide, being afterwards found in the brownish-yellow liquid. These substances must all be carefully distinguished from phylloxanthine, a product of decomposition of true chlorophyll.

Chlorophyll in animals.—The question as to the existence of chlorophyll in animals has been much debated. In attempting a solution of this question, the first error one must guard against is that of looking upon every green pigment as chlorophyll. In *Bonellia viridis* (a gephyrean worm), the colour is not due to chlorophyll at all, but to a somewhat similar pigment called Bonellein by Sorby.[4] In *Phyllodoce*

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[1] *Ann. de Pharm.* xxi. 261.

[2] *Proc. Roy. Soc.* xiii. 144.

[3] *Ibid.* xxxvi. 183.

[4] *Quart. J. Micros. Sci.* 1871, p. 166.

viridis (one of the polychæte worms), P. Geddes[1] failed to get any evolutions of oxygen on exposing it to sunlight. The reason is that the green pigment present, is not chlorophyll (MacMunn).[2] In other cases the formation of chlorophyll is due to parasitic algæ, existing within the animal organism, and is therefore not the direct product of the latter. There are cases, however, such as *Hydra viridis* and *Spongilla fluviatilis*, in which chlorophyll does exist in the cells of the animals themselves (Ray Lankester). MacMunn also has found it in several sea water sponges,[3] and in the elytra of cantharides beetles.[4] Poulton[5] has found it in the blood of many butterflies and moths, where it is probably derived directly from the food, and is apparently functionless. MacMunn[6] has found a chlorophyll in so-called livers of many invertebrates, which he terms entero-chlorophyll, and which he regards as being respiratory in function.

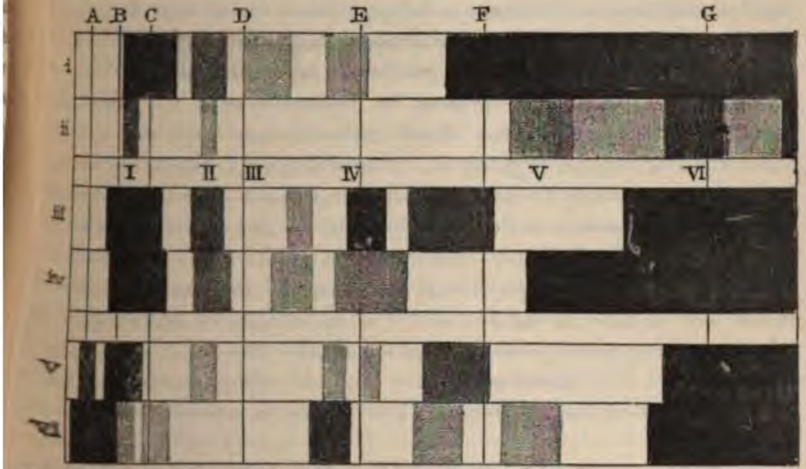

FIG. 11.—Absorption spectra of chlorophyll and its derivatives. i. Chlorophyll, strong solution. ii. The same much diluted to show the bands at the blue end of the spectrum. iii. Solution of phyllocyanin. iv. Solution of phylloxanthin. v. Product obtained by treating phyllocyanin with caustic alkali, then with acid, or by treating phyllotaonin with acid. vi. Ethyl compound of the preceding. (The above figure is from Dr. Schunck's article Chlorophyll in Watts' Dictionary.)

Tests for chlorophyll.—Obtain the pigment in solution and compare its absorption spectrum with that of chlorophyll. Add hydrochloric acid in large amount, and allow the mixture to stand some days ; filter off the dark deposit, dissolve some of it in ether, and compare the spectrum with that of phyllocyanin (*see* fig. 51, iii.); dissolve the rest in hot alkali; add excess of acetic acid; a precipitate is produced; shake up with ether to dissolve this, allow it to stand for several days; then compare the spectrum with that in fig. 51, v.

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[1] *Proc. Roy. Soc. Edin.* xi. (1881–2).

[2] *Journ. Marine Biol. Ass.* 1889, p. 59.

[3] *Journ. Physiol.* ix. 1.

[4] *Brit. Assoc. Rep.* 1883. This confirms the original statement of Pocklington which was called in question by Krukenberg and Chautard.

[5] *Proc. Roy. Soc.* 1885, no. 237.

[6] *Ibid.* xxxv. 370.

If these spectra are seen and identified, the colouring matter under examination is certainly chlorophyll.

Functions of chlorophyll.—Schunck concludes his memoir on chlorophyll by many interesting suggestions as to its chemical constitution. I here merely mention one of these. He considers that carbonic acid is one of the constituents of the molecule, but that it is held more loosely than in an ordinary carbonate, and yet in a state of greater condensation than it would be in a mere watery solution. It is thus in a favourable condition for transfer to the assimilating plasma, which effects its decomposition with elimination of oxygen, and the chlorophyll would then be in a state to take up fresh quantities of carbonic acid, acting therefore as a carrier of carbonic acid in the plant, just as hæmoglobin serves to convey oxygen in the animal economy.

Chlorophyll is always present in vegetable cells in which the formation of organic matter from carbonic acid and water, with elimination of oxygen, is going on. Parasitic plants like fungi obtain their nutriment ready formed from other organisms or decaying organic matter. They contain no chlorophyll, and do not decompose CO_2 as other plants do in the light. Plants grown in darkness from seeds or tubers contain no chlorophyll, and die when the food material stored in the seed or tuber is exhausted. The appearance of chlorophyll in etiolated plants on exposure to light indicates the commencement of assimilation. It is therefore certain that chlorophyll plays some part in the process of assimilation, but how it acts in assisting the process is unknown.

In the green cells of plants, the chlorophyll is found associated with proteid masses which it permeates and tinges green, forming the so-called chlorophyll corpuscles or granules, and probably the power of decomposing carbonic acid and water with evolution of oxygen resides in the chlorophyll corpuscle rather than in the pigment simply.

Jumelle[1] considers that chlorophyll assists in the transpiration of water from the leaves of plants.

[1] *Compt. rend. Soc. Biol.* 1889, p. 9.

CHAPTER XV

THE BLOOD

The blood forms a very convenient starting-point for a consideration of the chemistry of the elementary tissues. Using the word tissue in the sense of texture, some would, perhaps, hesitate to include the blood under that head ; but using the word in the sense of elementary principle, there seems but little reason why we should not include the blood with epithelium, muscle, &c., among the tissues. Many of the other tissues, such as muscle, are composed of a semi-fluid material, and there are but few parts of the body that do not contain a large percentage of water; blood is certainly the most fluid of the tissues, but floating in its fluid matrix are a large number of more solid particles, or blood corpuscles, which are analogous to the cellular elements of the other tissues. From an embryonic point of view, the blood is most nearly allied to the group of connective tissues : it is developed in connection with certain mesoblastic cells in situations where connective tissues are in process of formation, and its cellular elements are throughout life reinforced by the multiplication of cells which are situated also in various connective tissue structures (lymphoid tissue, marrow of bone).

The blood is not only distinguished from other tissues by its greater fluidity, but also by the fact that throughout life it is in continual movement. This movement constitutes what is called the circulation of the blood. Speaking generally, the functions of the blood consist in ministering to the needs of the other tissues. It receives oxygen from the air and conveys it to the tissues and organs generally ; it receives nutrient material from the alimentary canal, and this also it carries to the rest of the body. In return, it receives from the other tissues the products of their combustion, and conveys them to organs such as the lungs and kidneys, where they are finally got rid of or excreted.

The blood thus comes into relation with all the organs, and plays an important part in respiration, nutrition, and in all the other functions of the body.

In those animals in which there is but little or no differentiation of function, there is no circulating fluid to bring the different parts into relation with one another, and at the other extreme of the animal kingdom, where we find the greatest complexity, it is there also that we find the blood in its most highly developed condition. We shall find it most convenient to take the consideration of the blood in vertebrates first, and leave that of the different invertebrate classes for a subsequent chapter. This order will be the best, first, because our knowledge of vertebrate blood is more complete, and, secondly, from the point of view of human physiology and pathology it is of more practical importance.

Colour.—In vertebrates the blood is a somewhat viscous and, to the naked eye, homogeneous red liquid. The tint varies according to the state of oxygenation of the pigment hæmoglobin, to which the colour of the blood is due. The blood which leaves the lungs or, in aquatic animals, the gills, is of a bright scarlet hue, while that in the systemic veins is purplish. In contact with the air a loose combination called oxyhæmoglobin is formed which is scarlet, and in the tissues this oxygen is in great measure given up, and the blood returning to the heart has the darker purplish tint of hæmoglobin.

In only two vertebrate animals,[1] *Amphioxus*, or the lancelet, and *Leptocephalus*, another small fish, the blood contains no hæmoglobin, and is colourless.

Microscopic investigation of vertebrate blood shows that it is not a homogeneous red liquid, but that it consists of a nearly colourless liquid, the *plasma* or *liquor sanguinis*, holding in suspension large numbers of solid bodies—the corpuscles. These corpuscles are of two kinds—the coloured and the colourless. It is in the former, the coloured or red corpuscles, that the pigment hæmoglobin is contained.

The plasma, however, does in many cases contain a pigment in solution, or perhaps more than one. These will be treated of in connection with the serum.

Specific gravity.—Roy [2] has introduced a method for ascertaining the specific gravity of living blood. A drop of blood from the finger is received into a mixture of glycerine and water of known specific gravity. If the drop tends to rise or sink it is assumed that it is of lower or higher specific gravity than the fluid in which it is placed. By having ready to hand a number of such standard solutions of glycerine and water of different specific gravities, it is not difficult to find one in which the blood neither rises nor sinks, and, as its specific gravity is known, the specific gravity of the blood under examination is also ascertained. The average specific gravity of human blood thus found is 1060.

Lloyd Jones[3] finds that with a little practice this proceeding can be carried out very quickly, and from the examination of a large number of cases concludes that there is a 'diurnal variation' in the specific gravity of the blood, consisting of a fall during the day and a rise during the night. The specific gravity of the blood is higher in the male than in the female; and that during pregnancy, after exercise, or after the ingestion of food, there is a fall. In a passively congested part the specific gravity of the blood is high.

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[1] Lankester, *Proc. Roy. Soc.* vol. xxi. 1872, p. 71, *et seq.*

[2] Roy, *Proc. Physiol. Soc.* 1884.

[3] E. L. Jones, *Journ. of Physiol.* vol. viii. (1887), p. 1.

The specific gravity of defibrinated blood varies considerably, the average for human blood being 1055 (Becquerel and Rodier)[1]; for dog's blood, 1060 (Pflüger)[2]; for rabbit's blood, 1042 to 1052 (Gschleidlen).[3]

The specific gravity falls in anæmia and wasting diseases generally. It also falls after hæmorrhage.

The specific gravity of defibrinated blood may be ascertained by the use of the hydrometer, or more correctly by actual weighing (*see* p. 15).

Reaction.—The reaction of vertebrate blood is always alkaline. This is due to the alkaline salts which are present.

The demonstration of the alkalinity of the blood is very simple. A drop of blood is placed on the smooth, faintly reddened surface of a piece of dry, glazed litmus paper,[4] and after a few seconds is wiped off with a piece of clean linen rag moistened with water. The place where the blood has been standing is marked out as a well-defined blue patch (Schäfer[5]).

The manufacture of glazed litmus paper of the kind just alluded to renders unnecessary the somewhat elaborate methods adopted by older observers to demonstrate the alkalinity of the blood. Thus Kühne[6] placed the blood in a small dialyser suspended in a watch-glass full of water; some of the salts pass into the water, the alkalinity of which can be then shown. Liebreich[7] recommended porous slabs of plaster of Paris coloured by neutral litmus instead of litmus paper, and Zuntz[8] used litmus paper previously moistened with a strong solution of sodium sulphate or sodium chloride.

The alkalinity of plasma or of serum, where there is no difficulty arising from the presence of a mass of deeply coloured corpuscles, can be always demonstrated by the use of ordinary litmus paper, or of litmus solution.

Taste and odour.—The salts present in the blood give it a saline taste.

Blood has also a slight but peculiar odour dependent on the presence of minute quantities of volatile fatty acids. This odour, known as the *halitus sanguinis*, differs in different animals. It may be further developed by adding to the blood a mixture of equal parts of sulphuric acid and water.

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[1] Becquerel and Rodier, *Recherches sur les altérations du sang*, Paris, 1844. *Traité de chimie pathologique*, Paris, 1854, p. 41.

[2] Pflüger, *Pflüger's Archiv*, i. 75.

[3] Gschleidlen, quoted in Gamgee's *Physiological Chemistry*, p. 26.

[4] Such as are prepared by Messrs. Townson & Mercer, Bishopsgate Street.

[5] E. A. Schäfer, *Journal of Physiology*, vol. iii.

[6] Kühne, *Virchow's Archiv*, vol. xxxiii. (1865), p. 95.

[7] Liebreich, *Berichte d. deutschen chem. Ges. zu Berlin*, 1868, p. 48.

[8] Zuntz, *Centralbl. f. d. med. Wissensch.* 1867, no. 34.

Quantity of blood in the body.—This averages $\frac{1}{12}$ to $\frac{1}{14}$ of the total body weight. The most accurate method of estimating the total amount of blood in the body is Welcker's.[1] It may be briefly described as follows: A small quantity of blood is removed from the animal by venesection, defibrinated, measured, and diluted to known extents to serve as standards of comparison. The animal is then bled to death; the blood is defibrinated. The vessels are next washed out with water or saline solution, the washings added to the blood; lastly, the whole animal is finely minced with water or saline solution, the extract is filtered and added to the diluted blood previously obtained, and the whole is measured. The colour of the mixture is then compared with the standard solutions made from the few cubic centimetres of blood which were first removed, until one is discovered which has the same tint as the mixture. The amount of blood in the corresponding standard solution being known, the total quantity in the animal's body can in that way be easily calculated.

COAGULATION OF THE BLOOD

Within a few minutes after the blood has been shed it becomes viscous, and then rapidly sets into a solid red jelly. The formation of the jelly begins on the surface of the liquid and on the sides of the vessel in which it is contained; this rapidly spreads through the whole substance of the liquid. In a few minutes after this, drops of a more or less faintly straw-coloured liquid appear upon the upper surface of the jelly; these become larger and run together; the jelly shrinks from the sides of the containing vessel, more fluid collects, and ultimately the clot floats in a liquid. The appearance of this liquid, which is called *serum*, is due to the shrinking of one of the constituents of the clot, called *fibrin*, and the process of shrinking may go on for twelve to twenty-four hours. We can thus distinguish two steps in the coagulation of the blood:—

1. The stage of jellying.
2. The shrinkage of the clot, and the consequent expression of the serum.

With the microscope more details can be made out than with the naked eye. Filaments of fibrin are seen forming a network; many of these radiate from small clumps of blood tablets. These blood tablets

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[1] Welcker, *Zeitsch. f. nat. Med.* 3rd series, vol. iv. p. 147. *See* also Gschleidlen, *Physiol. Methodik*, 3te Lieferung, p. 337. *See* also Gamgee, *Physiol. Chem.* p. 215.

(Blutplättchen of Bizzozero) are minute colourless discs which occur in living blood, which disintegrate when blood is shed and which take, perhaps, some part in the formation of fibrin.

The fibrin filaments are exceedingly fine and straight; they entangle the blood corpuscles, and later, when they have contracted, the blood corpuscles to which they are attached are pulled out of shape (Ranvier).[1]

A convenient method for demonstrating the fibrin network is as follows (Schäfer)[2]:—

Place a drop of blood from the finger on to a slide, and cover it and put it aside for a quarter of an hour to coagulate. Then allow a drop of a solution of borax-carmine or logwood to run under the cover-glass. This decolorises the red corpuscles, and stains the nuclei of the white corpuscles, the fibrin filaments, and the blood tablets. The preparation may be rendered a permanent one by allowing a drop of dilute glycerine to diffuse into the fluid, and cementing the cover-glass with gold-size.

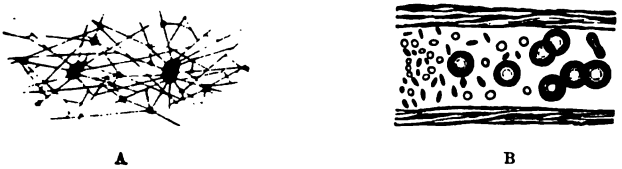

FIG. 52.—Fibrin filaments and blood tablets. A, network of fibrin shown after washing away the corpuscles from a preparation of blood that has been allowed to clot. Many of the filaments radiate from small clumps of blood tablets; B, (from Osler) blood corpuscles and blood tablets within a small vein.

Microscopic examination thus shows that fibrin is formed from the blood plasma, and that the clot consists of fibrin with the blood corpuscles entangled in its meshes. Fibrin can also be obtained from plasma when the corpuscles have been removed from it, as will be explained fully later on. Serum is plasma *minus* fibrin. The relation of plasma, serum, and clot can be seen at a glance in the following scheme of the constituents of the blood:—

Blood	Plasma	Serum	
		Fibrin	Clot or Crassamentum
	Corpuscles	red	
		white	
		blood-tablets	

It may be roughly stated that in 100 parts by weight of blood 60–65 parts consist of plasma and the remaining 35–40 of corpuscles.

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[1] Ranvier, *Traité technique d'histologie*, p. 214.
[2] Schäfer, *Essentials of Histology*, 2nd edit. 1887, p. 7.

Rapidity of coagulation.—The following table gives the average time after the shedding of the blood that coagulation commences (Nasse):—

Blood of	fowl	begins to	coagulate in	1½ minute
,,	pig	,,	,,	½ to 1½ minute
,,	sheep	,,	,,	½ to 1½ ,,
,,	rabbit	,,	,,	½ to 1½ ,,
,,	dog	,,	,,	1 to 3 minutes
,,	man	,,	,,	3 to 4 ,,
,,	horse and ox	,,	,,	5 to 13 ,,

In man, solidification is completed in 9 to 11 minutes, but rather sooner in the case of women.

In cold-blooded animals, coagulation is rather slower than in mammals, the resulting clot is small after it has shrunk, and the quantity of serum formed is correspondingly large. In birds, not only is coagulation very rapid, but the clot is proportionately large, and the yield of serum small.

The yield of serum from sheep's blood is greater than from that of other common mammals. When large quantities of serum are needed, it is therefore best to use the blood of this animal.

The buffy coat, or Crusta phlogistica.—If the blood coagulates slowly, as does that of the horse, or that obtained from persons suffering from acute inflammatory diseases like pneumonia, the corpuscles will have time to sink before the formation of fibrin has begun. The red corpuscles being heavier than the white sink more rapidly, and thus the upper stratum of the clot consists chiefly of fibrin and white corpuscles. It is, therefore, not so red as the lower portions of the clot, and it is termed the buffy coat. The buffy coat is generally cupped; this is because the fibrin contracts more in the centre than at the sides, where it adheres to the interior of the containing vessel, the comparative absence of corpuscles rendering more evident also the shrinking which is so characteristic of fibrin.

In conditions of anæmia and chlorosis the formation of a buffy coat has been also observed in the shed blood. This does not seem to be due to coagulation being very slow, but rather to the subsidence of the red corpuscles being very quick on account of the low specific gravity of the plasma.

Another phenomenon may also be sometimes observed in blood and similar fluids that clot slowly. Clotting occurs, and a fluid, apparently serum, is squeezed out; but it is not serum, as in a short time it also clots; the fluid is, in fact, plasma, in which the process of fibrin formation has either not yet occurred or has only partially taken place.

Other changes accompanying coagulation.

1. In temperature; there is a slight rise in temperature.

2. In reaction; the alkalinity diminishes.

3. In electrical potential. Hermann states the coagulated parts are negative to the non-coagulated parts.

4. The amount of oxygen in the blood is diminished. The interior

of a blood-clot will be always found dark in comparison with the exterior. This seems to be because the still living blood cells are undergoing chemical changes, and derive the necessary oxygen from the oxyhæmoglobin, which consequently becomes reduced and dark.

5. The tension of carbonic acid in the blood rises.[1]

6. Traces of ammonia are stated by Richardson to be given off, and this he erroneously supposed to be the cause of the coagulation. But blood collected over mercury coagulates, and in this case there is no escape of ammonia.

The coagulation of the blood is hastened by the following means :—

1. A temperature a little above that of the body (Hewson).[2]

2. Contact with foreign matter ; thus threads, wire, &c., introduced into the living blood vessels will cause a clot to form, starting from the object introduced. The diseased wall of a blood vessel acts in the same way, so also does fibrin already deposited on the vascular wall. When blood is shed, it clots first in those parts situated next to the wall of the vessel in which it is contained.

3. Agitation ; this really is the same thing as repeated contact with foreign matter.

4. Dilution with not more than twice its volume of water.

5. The addition of minute quantities of neutral salts, such as sodium chloride. Calcium salts are necessary for the efficient formation of fibrin, and for the activity of the fibrin ferment (Green,[3] Ringer[4]).

The coagulation of the blood is hindered or prevented by the following means :—

1. A low temperature ; when blood is received into a vessel cooled by ice to 0°C., the blood remains uncoagulated for an hour or more.

Davy[5] stated that blood can be frozen and thawed several times in succession without losing its power of coagulating. There is probably, however, a small quantity of fibrin formed with every thawing ; not sufficient to cause jellying throughout the mass of blood. With plasma from which the greater number of corpuscles have been separated, it is easy to demonstrate this, as the few shreds of fibrin which are formed can be readily seen in such plasma, while they are obscured by the opacity of blood. Their formation is probably due to the effect of the crystals of ice breaking up a certain number of white corpuscles, and so liberating a quantity of fibrin ferment, which causes a formation of fibrin when the temperature is sufficiently raised.[6]

2. The addition of a sufficient quantity of neutral salt. Hewson employed sodium sulphate for this purpose. Magnesium sulphate is

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[1] Strassburg, *Pflüger's Archiv*, vi. 65.

[2] Hewson's Works edited by Gulliver, Sydenham Soc. London, 1846.

[3] Green, *Journ. of Physiol.* viii. 354. [4] *Proc. Physiol. Soc.* Feb. 1890.

[5] Dr. John Davy, *Anat. and Physiol. Researches*, London, 1859.

[6] Halliburton, *Proc. Roy. Soc.* vol. xliv. (1888), p. 266.

also now largely employed. Sodium chloride, potassium nitrate, and several other salts act similarly. When blood is received into an approximately equal volume of saturated solution of sodium sulphate, or of a 10 per cent. solution of sodium chloride, or into a quarter of its volume of a saturated solution of magnesium sulphate, coagulation may be indefinitely postponed. When, however, such a mixture of blood and salt is diluted considerably with water, coagulation ensues, as the inhibitory influence, which the strong salt solution exerted on the formation of fibrin, is removed.

3. Contact with living vascular walls. After death the blood remains fluid in the smaller vessels for many hours. Brücke (1857) kept the blood fluid in the interior of a tortoise's heart (removed from the body) for eight days. A more convenient vessel is the jugular vein of a large animal like the horse. If the vein be ligatured in two places, so as to include a quantity of blood within it, and then removed from the animal, the blood will remain fluid for hours or even days, provided that the vessel be hung in a cool place, and that the inner lining of the vein preserves its integrity for that time. If the vein be opened and the contents be allowed to come into contact with foreign matter, coagulation will ensue in a few minutes. This experiment, often spoken of as the 'living test-tube experiment,' has been employed in the researches of Hunter,[1] Hewson,[2] Lister[3] and Fredericq.[4] If the double ligature be applied antiseptically, and the vessel be allowed to remain in the body, the wound heals, and the included blood-column will be found uncoagulated months afterwards (Baumgarten,[5] Senftleben[6]). Blood, however, which escapes from the vessels into the tissues soon coagulates.

4. Injection of peptone into the circulation. If a certain quantity of commercial peptone, such as Witte's or Grübler's, be injected into the circulation, and the animal then killed by bleeding, the blood will be found to have lost the property of coagulating (Schmidt-Mulheim,[7] Fano[8]). The blood becomes normal again about three hours after the injection. The dose for a dog is 0·3 gram of peptone for every kilogram of body weight. In rabbits peptone has no such effect in hindering coagulation. Pollitzer[9] has shown that this property of so-called peptone is really due to the albumoses of which commercial preparations of 'peptone' chiefly consist, and especially to one of these called hetero-albumose. Pure peptone has little or no such effect. He has also

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[1] Hunter, *Works*, vol. iii. p. 29.

[2] Hewson, *Works*, p. 22.

[3] Lister, *Proc. Roy. Soc.* vol. xii. (1863), p. 580.

[4] Fredericq, *Recherches sur la constitution du plasma sanguin*, Gand, 1878.

[5] *Cohnheim's Pathology*, New Syd. Soc. 1889, p. 177.

[6] *Virchow's Arch.* lxxvii. 421

[7] Schmidt-Mulheim, *Du Bois Reymond's Arch. f. Anat. und Physiol.* 1879.

[8] Fano, *Ibid.* 1881, p. 277.

[9] Pollitzer, *Journ. of Physiol.* vii. 282

shown that these albumoses delay the coagulation of the blood after it is shed. They also cause a similar delay in the clotting of dilute salted plasma.[1] Various diastatic ferments injected into the blood stream act in the same way (Salvioli[2]).

In all these cases the presence of peptone, either free or when injected into the blood stream, possibly in a loosely combined condition,[3] acts in all probability by inhibiting the activity of the fibrin-ferment. This inhibitory influence can be removed by passing a stream of carbonic acid through the blood.

5. Contact with oil. When blood is surrounded by fluid, of a surface-tension different from its own, and which does not mix with it, its coagulation is much delayed. Thus Freund found that if he smeared a glass vessel with vaseline, and carefully received blood into it through a greased cannula in direct communication with the artery of an animal, he could by covering the blood so obtained with a layer of liquid paraffin, keep it from coagulating for several hours. Haycraft[4] obtained a similar result by allowing blood to drop through a layer of liquid paraffin on to greased mica plates. Haycraft and Carlier[5] received blood directly from the finger tip into a tall cylindrical vessel filled with castor oil, which is very viscid. The drops sink slowly through the oil, and by occasionally inverting the vessel, the blood may be kept from coming in contact with its ends for a considerable time. The microscopic characters of uncoagulated blood may be examined on a greased slide, if great care be taken to prevent the blood from coming in contact with anything solid or ungreased after it is shed.

6. Addition of small quantities of caustic alkalis or ammonia. In these cases the reagent used is, however, so strong as to alter very considerably the natural condition of the constituents of the blood.

7. Addition of acetic acid or excess of carbonic acid. There is here again the objection to the use of a strong acid like acetic acid, which readily converts globulins like fibrinogen into acid-albumin. Passing a stream of carbonic acid through the blood after dilution precipitates the fibrinogen, and so of course prevents the formation of fibrin. The greater quantity of carbonic acid in venous as compared with arterial

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[1] Halliburton, *Proc. Roy. Soc.* vol. xliv. (1888), p. 264.

[2] Salvioli, *Centralbl. f. d. med. Wiss.* 1885, p. 913.

[3] It is difficult to find 'peptone' after injection into the blood, perhaps because it is, as Hofmeister considers, held in combination by the white corpuscles. Neumeister has, however, shown that after a short time these substances (albumoses and peptones) are excreted by the kidneys, so they must be present as such in the blood, or only very loosely combined for a time at least. Further remarks on the presence and fate of peptones in the blood will be found in the Chapter on Absorption.

[4] Haycraft, *Proc. Roy. Soc. Edin.* July, 1887.

[5] Haycraft and Carlier, *Brit. Med. Journ.* vol. ii. (1888), p. 229.

blood is said to be the cause of the slower coagulation observed in the former.

8. Heating blood to 56°C. immediately it is withdrawn from the body also prevents any formation of fibrin, as the temperature mentioned is sufficiently high to cause a heat-coagulum of the proteid fibrinogen, the fibrin precursor.

9. The addition of an equal volume of a 0·5 per cent. solution of cane sugar delays the coagulation of blood for about an hour (J. Müller[1]). The addition of other viscous substances, such as egg-albumin or glycerine, has the same effect.

10. The addition of much water to the blood delays its coagulation.

11. A watery extract of the medicinal and common leech prevents the coagulation of the blood (Haycraft[2]). This is interesting in view of the difficulty often experienced in controlling hæmorrhage from leech bites; the secretion from the leech evidently prevents the formation of a clot, which usually performs the part of a plug in small wounds of this kind.

12. Blood which naturally clots slowly or not at all.

(*a*) The blood of embryo fowls does not coagulate before the 12th or 14th day of incubation.[3]

(*b*) In certain morbid conditions the blood also clots slowly (*see* Chapter XVI).

So far then we have described the naked eye and microscopic phenomena of coagulation; we have enumerated the various conditions which hasten or delay clotting; we have arrived at the conclusion that the essential fact in the process is the separation of the substance fibrin from the plasma, and it has been incidentally mentioned that the formation of fibrin is due to the activity of an organised ferment[4] called the fibrin-ferment.

It will be now convenient to take up the substances fibrin, plasma, and serum, and discuss their properties more fully. After we have considered at greater length the properties of the constituents of the plasma, it will be easier to understand the theory of the cause of the coagulation which is now generally held, and the grounds upon which it rests. The theory is that of Hammarsten, and may be briefly stated as follows :—

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[1] J. Müller, *Poggendorff's Annalen*, xxv. 540.

[2] Haycraft, *Proc. Physiol. Soc.* 1884, p. 13.

[3] Tiegel has stated that the blood of certain snakes does not clot for many hours after being shed; but other observers have not found any such delay in the coagulation (see *Journ. of Physiol.* vii. 322).

[4] By an unorganised ferment is meant a chemical agent which produces certain changes in the materials with which it comes into contact, without itself undergoing any change. The term organised ferment is applied to those which, like yeast and bacteria, consist of living organisms.

When the blood is within the vessels, one of the constituents of plasma, a proteid of the globulin class called fibrinogen, exists in a soluble form. When the blood is shed, fibrinogen is converted into the comparatively insoluble substance fibrin. This change is brought about by the activity of a special unorganised ferment called the fibrin-ferment. This ferment does not exist in healthy blood contained in healthy blood vessels, but is one of the products of the disintegration of the white corpuscles and probably also of the blood tablets, that occurs when the blood leaves the vessels, or comes into contact with foreign matter.

The Plasma or Liquor Sanguinis

The liquid in which the corpuscles float can be obtained by employing one or other of the methods already described for preventing the blood from coagulating. The corpuscles have a higher specific gravity than the plasma ; they therefore sink, and the supernatant plasma can be removed by a pipette or siphon. It may then be more thoroughly cleared from corpuscles by the use of a centrifugal machine (*see* p. 17).[1]

The following are the forms of plasma that may be obtained :—

a. Pure plasma.—This may be obtained from horse's veins by what has already been described as the living test-tube experiment. The plasma removed from the top of the vein clots slowly, at the temperature of the air (usually in 15–30 minutes) ; that deeper down, nearer to the corpuscular sediment, itself contains more corpuscles, and coagulates more rapidly. In all cases the plasma coagulates more quickly at a temperature of 40° C. The process of clotting consists of the same stages as the clotting of blood already described ; the clot itself consists of pure fibrin, or fibrin with only a slight admixture of corpuscles.

b. Cooled plasma.—This is another form of pure plasma. Blood is allowed to flow into the middle compartment of a vessel containing three concentric chambers, the inner and outer of which are filled with ice (Burdon Sanderson).[2]

The corpuscles settle ; the plasma can be removed, and it is found to possess the same characters as those already mentioned.

c. Transudation fluids.—Pericardial and hydrocele fluids, and similar transudations into serous cavities, resemble pure plasma very closely in composition. As a rule, however, they do not coagulate spontaneously ; but after the addition of fibrin-ferment (or liquids like serum which contain fibrin-ferment) they always yield fibrin. In cases, however, in which the serous sac is inflamed, as in pericarditis, the fluid is found to contain a large number of white corpuscles; it then clots after removal from the body without the addition of any ferment.

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[1] In the case of pure plasma, the tubes during centrifugalising must be kept cool by enclosing them in larger tubes filled with powdered ice.

[2] *Handbook for the Physiological Lab.* p. 168.

d. Salted plasma.—This may be obtained by mixing the blood immediately when shed with the necessary amount of strong saline solution (p. 224). This is found better than Hewson's original method, in which he stirred the blood with the solid salt (sodium sulphate). The corpuscles settle somewhat slowly in the case of the blood of oxen and sheep, but more rapidly in that of the horse. After twenty-four hours' standing, a good supply of salted plasma can be generally obtained readily. This settling can, however, always be hastened by the centrifuge. In sodium sulphate plasma, a few strands of fibrin may sometimes form, but as a rule it remains many days unaltered. On diluting it with four or six times its volume of water, coagulation ensues slowly; fibrin often not appearing for many hours, but always more quickly at a temperature of 40° C. and always within a few minutes after adding fibrin-ferment. Sodium chloride plasma acts similarly. Magnesium sulphate plasma is preferred by some workers, because, when diluted simply with water, it clots more slowly than other forms of salted plasma, or sometimes not at all. It, however, never fails to clot rapidly with fibrin-ferment; on this account it is generally employed as a test for this ferment.

Salted plasma when diluted with water, without the addition of fibrin-ferment, clots because the dilution renders the proportion of salt present, insufficient to inhibit the activity of the ferment; the ferment is present in small quantities, either from the presence of some few corpuscles not removed by the process of centrifugalising, or because some of these corpuscles have already undergone disintegration.

Diluted magnesium sulphate plasma is least prone to undergo this slow spontaneous clotting, because the proportion of salt used has precipitated some of the precursor of fibrin, fibrinogen, and perhaps other globulins as well; this precipitate is a flocculent one, and settles with the corpuscles to the bottom of the vessel. The proportion of this salt usually employed is that recommended by Hammarsten,[1] four parts of blood to one of saturated solution of magnesium sulphate. Magnesium sulphate plasma therefore possesses the disadvantage of not containing all the proteid matter of the original plasma; a minor disadvantage in its use is that it is often stained with hæmoglobin.[2]

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[1] Hammarsten, *Pflüger's Archiv*, xiv. 220.

[2] Hamburger (*Zeit. Biol.* xxvi. 414) has shown that when blood is mixed with salt solutions of different concentrations, there is for each salt a certain concentration when no hæmoglobin is dissolved out from the corpuscles, while a saline solution of less concentration becomes tinged with the pigment. The mean of these two limits gives

The process of clotting in all these forms of salted plasma leads to the formation of fibrin, and ultimately to the expression of a mixture of serum and saline fluid, which may be called salted serum.

e. Syrupy plasma.—This may be obtained by filtering blood in which the coagulation has been delayed, by mixing it when shed with its own volume of a 0·5 per cent. solution of cane sugar. It coagulates in from 30 to 60 minutes.

f. Peptone plasma.—This may be obtained by centrifugalising peptone blood, the preparation of which has been already described. The inhibitory influence of the peptone is removed by passing through the plasma a stream of carbonic acid gas[1] (Fano), or by the addition of lecithin (Wooldridge). On cooling this form of plasma to 0° C. a proteid precipitate consisting of rounded granules occurs (Wooldridge). Such a precipitate produced by cooling is not obtained in pure plasma, nor in salted plasma.

g. Bile-salt plasma.—This form of plasma obtained by mixing blood and a certain proportion of bile salts has been used by Nauck and Samson-Himmelstjerna.[2] These observers find that with this form of plasma the accelerating influence of small quantities of lecithin and of many other organic substances (glycocine, uric acid, &c.) on coagulation can be demonstrated. Conclusions based, however, on forms of plasma where there has been an admixture with complex organic substances, like bile salts or commercial peptone, should be received with caution, unless supported by corroborative experiments with pure plasma.

h. Leech plasma.—This form of plasma is obtained from blood in which coagulation has been prevented by mixing it with extract of leeches.

General Characters and Composition of the Plasma

The plasma is alkaline in reaction, and yellowish in colour; in the case of man, its specific gravity varies between 1026 and 1029.[3] Like the blood it clots, and the process of clotting leads to the formation of

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numbers identical with the isotonic coefficients of de Vries (Pringsheim's *Jahrb. wiss. Botanik*, xiv. Heft iv.)

[1] In connection with this action of carbonic acid gas, it should be noted that Lahousse (*Archiv f. Physiol. Anat.; Physiol. Abth.* 1889, p. 77) has found that 'peptone blood' contains only about half the normal quantity of this gas; the oxygen is rather higher in amount in peptone blood than in normal blood. Bohr (*Centralbl. f. Physiol.* 1888, no. 11) finds that in a dog into which either peptone or leech infusion has been injected, the output of carbonic acid in the expired air is much diminished.

[2] *Inaugural Dissertations*, Dorpat, 1882 and 1886.

[3] Gamgee, *Physiological Chemistry*, p. 34. Gautier, *Chimie appliquée à la physiologie*, 1874, vol. i. p. 489. C. Schmidt (*see* Gamgee, p. 127) gives the specific gravity of healthy plasma rather higher, viz. 1031.

fibrin ; this process can be hindered or hastened by the same agencies as have already been mentioned in the case of blood.

The plasma consists largely of water ; the solids, dissolved in it, fall into three classes : proteids, extractives, and inorganic salts.

Gamgee[1] gives the following table, deduced from the observations of C. Schmidt and Lehmann, which shows the relations of these substances to one another in human liquor sanguinis.

1,000 parts of blood yield { 513·02 parts of corpuscles
{ 486·98 parts of plasma

1,000 parts of plasma contain :

Water	902·90
Solids	97·10
Proteids—1. yield of fibrin	4·05
2. other proteids	78·84
Extractives, including fat	5·66
Inorganic salts	8·55

In round numbers it may be stated that the plasma contains 10 per cent. of solids, of which about 8 per cent. are proteid in nature.

Besides these solids, the plasma has dissolved in it certain gases (oxygen, carbonic acid, and nitrogen), the consideration of which will be taken with respiration.

General Characters of the Serum

Serum may be obtained by either of the following methods:—

1. By allowing plasma to coagulate ; the clot is filtered off ; the filtrate is serum.

2. By allowing blood to coagulate. This is the method more usually employed. The fluid squeezed out by the contraction of the clot is serum, which may be removed by a pipette or siphon ; and then completely freed from corpuscles by the use of the centrifugal machine.

The serum has the same colour and, approximately, the same specific gravity as the plasma. It is rather more alkaline than the plasma from which it has separated. It however does not clot spontaneously. In other words, the essential difference between it and plasma is, that in serum the fibrin-yielding constituent of the plasma (fibrinogen) has been removed in the form of fibrin. Serum also contains the disintegration products of white corpuscles and blood tablets, of which the two most important are globulin and fibrin ferment.

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[1] Gamgee, *Ibid.* p. 128.

Serum contains the same three classes of constituents as the plasma, viz. proteids, extractives, and salts. The extractives and salts are the same in the two liquids. The proteids are different, as is shown in the following table :—

PROTEIDS OF

Plasma	*Serum*
Fibrinogen (subsequently changed to fibrin) .	Serum-globulin
Serum-globulin	Serum-albumin
Serum-albumin	Fibrin-ferment

We have now to take up these different proteids one by one. The fibrin-ferment has been included among the proteids for reasons which will be fully dealt with later on.

Fibrin

The microscopic characters of fibrin have been already described. A supply of fibrin in sufficient quantity for chemical investigation may be obtained

(1) By allowing plasma to coagulate.

(2) By allowing lymph to coagulate. The amount of fibrin formed from lymph is 0·4 to 0·8 per 1,000.

(3) By inducing coagulation in fluids which contain fibrinogen ; such for instance as hydrocele fluid, pericardial fluid, and exudations into other serous sacs. This may be done by adding fibrin-ferment to such fluids.

(4) By the addition of fibrin-ferment to solutions of pure fibrinogen.

(5) By whipping freshly-drawn blood with a bunch of twigs ; the fibrin adheres to the twigs, and entangles but few corpuscles ; it may then be washed by a stream of running water. This is the usual method by which fibrin is obtained. The amount of fibrin formed from human blood is 2·2 to 4 per 1,000.

Fibrin is a white stringy solid when fresh ; when dried it becomes greyish in appearance. It is extensible and elastic ; and it is owing to the retractility of its fibres that a blood clot contracts.

It is exceedingly difficult to prepare fibrin free from white blood corpuscles ;[1] and certain constituents of those corpuscles are nearly always adherent to it. Even after prolonged washing with water, there is always a considerable quantity of fibrin-ferment adherent to it.

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[1] A stringy substance may be obtained from white corpuscles which is not true fibrin. It is a nucleo-albumin.

It may be purified by repeated extraction with alcohol and ether. It has then the following elementary composition:[1] C, 52·68; H, 6·83; N, 16·91; S, 1·1; O, 22·48 per cent. Fibrin is a proteid, though it is not nearly so soluble in neutral liquids as most proteids are.

Like many other organic substances fibrin decomposes solutions of hydric peroxide.

It is soluble with difficulty in a 6 per cent. solution of potassium nitrate; in 5–15 per cent. solutions of sodium chloride; in 5–10 per cent. solutions of magnesium sulphate; and in similar solutions of other neutral salts, such as sodium sulphate and ammonium sulphate. This occurs most readily at a temperature of 40° C.

Denis described three varieties of fibrin: (1) fibrine concrète modifiée; (2) fibrine concrète globuline; and (3) fibrine concrète pure. Hammarsten has repeated these observations of Denis, and, in the main, confirms his conclusions:[2] the first variety is that obtained from arterial blood, and is ordinary fibrin; the second variety is the slimy substance which forms when a 10 per cent. solution of sodium chloride is added to certain varieties of fibrin; it is, in fact, not true fibrin at all, but a nucleo-albumin which swells up with sodium chloride, and which is contained in white blood corpuscles, and which we shall have to describe fully with the white corpuscles; this form of fibrin (so-called) is obtained whenever white corpuscles or pus corpuscles are present in excess. The third variety of fibrin is that which forms in venous blood, and this dissolves more readily in saline solutions than that obtained from arterial blood.

The substance which goes into solution when fibrin is dissolved in saline solutions is undoubtedly a proteid of the globulin class. It is coagulated by heat, it is precipitated from its solutions by saturating them with magnesium sulphate, and also by dialysing away the salt from such solutions. This globulin, however, cannot be reconverted into fibrin by the addition of fibrin-ferment. The temperature of heat coagulation is 60°–65° in a sodium chloride solution; 73°–75° in a magnesium sulphate solution.

If putrefaction is not prevented by the addition of a few crystals of thymol or some other antiseptic, there is a more rapid and thorough solution of the fibrin. Indeed, some observers have supposed that fibrin never dissolves in saline solutions, except by the process of putrefaction. Salkowski[3] has shown that a ferment, the result of bacterial life, can be actually separated from the microbes; this acts upon fibrin like trypsin, producing first globulins, then albumoses, and finally peptones.

Weak hydrochloric acid (0·2 per cent.) causes fibrin to swell up into a transparent jelly. Stronger acids dissolve it in time with the formation of acid-albumin or syntonin, and albumoses.

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[1] Hammarsten, *Pflüger's Archiv*, xxii. 484.

[2] *Ibid.* xxx. 437.

[3] Salkowski, *Zeitschr. Biol.* xxv. 92.

Digestive ferments act readily on fibrin, so that fibrin is a convenient substance to use when demonstrating the activity of the digestive juices. Pepsin in an acid solution, and trypsin (from the pancreas) in an alkaline solution, cause first a splitting of the fibrin into two globulins, one coagulating at 56°, the other at 75° C.; then the formation of albumoses and peptones.

The subject of the solubilities of fibrin has been worked at by numerous experimentalists. The following is a résumé of the different views that have been held:—

The researches of Denis and of Hammarsten have been already referred to. Gautier[1] (1874) speaks of the proteid which goes into solution as an albumin. Hoppe-Seyler[2] has shown that it is a globulin, but considers that putrefaction plays an important part in the process of solution. Otto[3] also states that the substance in solution is of the nature of paraglobulin (now more commonly known as serum-globulin). Green[4] has (carefully preventing putrefaction) demonstrated that the globulin in solution is not exactly like either of the two globulins of the plasma (fibrinogen and serum-globulin), but that it is in reality a mixture of two new globulins—one soluble, the other insoluble in 1 per cent. sodium chloride solution.[5]

I[6] have shown that the discrepancies in the heat-coagulation temperature, as noted by various observers, are due to the different salts used for dissolving the fibrin.

The solubilities of fibrin have been discussed from another point of view, viz. as a source of fibrin-ferment, by Gamgee[7] and by Lea and Green.[8]

The solubilities of fibrin in digestive fluids have been a subject of investigation in all recent researches on digestion (Kühne and Chittenden, &c. &c., *see* Digestion). It may, however, be here conveniently mentioned that the splitting of fibrin into two globulins, which is antecedent to the formation of digestive products proper (albumoses and peptones), was discovered in the case of gastric digestion by K. Hasebroek,[9] and in that of pancreatic digestion by A. Herrmann.[10] These are not formed in the digestion of other proteids, nor in fibrin which has been previously boiled or coagulated by alcohol.

Estimation of fibrin.—The amount of fibrin in blood may be ascertained in the following way:

The fibrin is obtained by whipping a known quantity of blood; it is well washed and kneaded under a tap, and finally with distilled water and alcohol; it is then collected, dried, and weighed on a filter of known weight. Lastly it is incinerated and the weight of the ash deducted.

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[1] Gautier, *Comptes rendus*, 1874, vol. ii. p. 227.

[2] Hoppe-Seyler, *Physiol. Chem.* p. 417.

[3] Otto, *Zeit. physiol. Chem.* viii. 129.

[4] Green, *Journ. of Physiol.* viii. 372.

[5] It is possible that one of these may result from the solution of the fibrin itself, and the other from that of the entangled white corpuscles.

[6] Halliburton, *Journ. of Physiol.* viii. 149; ix. 234.

[7] Gamgee, *Ibid.* ii. 145.

[8] Lea and Green, *Ibid.* iv. 380.

[9] K. Hasebroek, *Zeitschr. physiol. Chem.* xi. 348.

[10] A. Herrmann, *Ibid.* p. 508.

In liquids like pericardial fluid, which do not coagulate spontaneously, a small quantity of serum or of an active solution of fibrin-ferment must be added, and then the fibrin collected, washed, and weighed after coagulation has occurred.

In blood which has already coagulated, the washing of the fibrin free from corpuscles is a long and troublesome process.

In making comparative experiments of the amount of fibrin in two liquids, say in two specimens of pericardial fluid, instead of weighing the fibrin it may be stained with carmine, and then subjected to the action of equal amounts of artificial gastric juice at 40° C. The fibrin dissolves and the carmine passes into solution. The liquid most deeply coloured is that which had the most fibrin in it. The relative amount of fibrin in the two liquids may then be ascertained by finding out how much it is necessary to dilute the more deeply coloured one until it has the same tint as the other.

The fibrin factors.—A. Schmidt considered that fibrin was formed as a union of fibrinogen and fibrino-plastin (now called serum globulin), and that this union was accomplished by the activity of the fibrin ferment. The two substances fibrinogen and 'fibrino-plastin' were therefore termed the fibrin factors.

Hammarsten has, however, shown that Schmidt's fibrino-plastin takes no part in the formation of fibrin, but that fibrinogen is the only fibrin factor, or fibrin precursor in the plasma.

The term fibrin factor might very conveniently be dropped altogether, as the word fibrinogen has precisely the same meaning.

Fibrinogen

This may be prepared from plasma in the following ways:

1. The plasma is diluted with 15 times its volume of cold water, and a stream of carbonic acid gas passed through it; a precipitate of serum-globulin is obtained, and filtered off; the plasma is then further diluted and again a stream of carbonic acid passed through it; a further precipitate occurs which consists of fibrinogen. This is Schmidt's method; it is, however, one which causes only an incomplete and imperfect separation of fibrinogen from the plasma.

2. Hammarsten's method depends on a property in which fibrinogen differs from serum-globulin, in being completely precipitated from its solutions, by half saturation with sodium chloride, i.e. by mixing the solution with an equal quantity of saturated solution of sodium chloride.

The precipitate so obtained is washed with a half-saturated solution of the salt, then dissolved in a 6–8 per cent. solution of the same salt, and again precipitated by half saturation. These operations should be performed rapidly, as prolonged contact with a half-saturated solution of sodium chloride renders the precipitate of fibrinogen very insoluble. The precipitate finally obtained is apparently soluble in water; but it is enabled to dissolve in water by means of the salt adhering to it. By this method fibrinogen may be prepared, not only from plasma and lymph, but also from exudation fluids such as pericardial and hydrocele fluids.

Fibrinogen so obtained is found to have the properties characteristic of globulins, viz. insolubility in pure water, solubility in water containing oxygen, and in weak solutions of neutral salts. It is precipitated from such solutions by dialysing away the salt, or by increasing the concentration of the salt beyond a certain point, in the case of sodium chloride up to half saturation, i.e. about 18 per cent.

The characteristic properties of fibrinogen are:

1. In the presence of minute quantities of certain salts of which sodium chloride and calcium sulphate seem to be the most important, the addition of fibrin-ferment causes the formation of fibrin. Without such addition, a solution of fibrinogen prepared by Hammarsten's method will remain uncoagulated indefinitely.

2. It enters into the condition of a characteristically sticky heat-coagulum at the very low temperature of 56° C. This is true, not only with regard to solutions of pure fibrinogen; but that a heat-coagulum is formed at the same temperature in pure plasma obtained by the living test-tube experiment is a very striking proof that fibrinogen is present as such in the blood.[1] This was first shown by Hewson,[2] and the fact was subsequently rediscovered by Fredericq.[3]

Hammarsten[4] showed that in the formation of fibrin from pure fibrinogen, as well as during the process of heat-coagulation, not only is there a formation of a solid clot, but simultaneously a small quantity of a proteid (a globulin coagulating at a temperature of 65° C.) enters into solution, which is probably a decomposition product of the fibrinogen molecule.

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[1] This fact cannot, however, be regarded as absolute proof that fibrinogen is present as such in the circulating blood. Injection of fibrin-ferment into the circulation does not necessarily cause intravascular clotting; it is therefore possible that certain counteracting agencies prevent fibrin being formed in healthy circulating blood, or that the fibrin when formed is immediately redissolved. Wooldridge supposes that in the circulating blood a precursor of Hammarsten's fibrinogen is present, and that this is readily changed into fibrinogen when the blood is shed.

[2] Hewson, *Works* edited by Gulliver, p. 26.

[3] Fredericq, *Recherches sur la constitution du plasma sanguin*, Gand, 1878.

[4] Hammarsten, *Pflüger's Archiv*, xxii. 480.

3. The fact that half-saturation with sodium chloride will completely precipitate fibrinogen from its solutions is important, as it enables us to separate it from serum-globulin, to which it is so similar in many particulars.

4. The fact that it is necessary to dilute plasma to a greater extent than in the case of serum-globulin, in order to precipitate it by a stream of carbonic acid, is also characteristic.

5. Its specific rotatory power for yellow (i.e. sodium) light is 43° (Herrmann[1]). The opalescent character of solutions of fibrinogen, however, renders polarimetric observations difficult.

6. Its percentage elementary composition is as follows: C, 52·93; H, 6·9; N, 16·16; S, 1·25; O, 22·26 (Hammarsten[2]); i.e. there is a slightly higher percentage of carbon, hydrogen, and oxygen than in the fibrins which is formed from it.

Estimation.—The quantity of fibrinogen in a solution may be approximately estimated by weighing the washed and subsequently dried fibrin obtained by the addition of fibrin-ferment, or by similarly drying and weighing the precipitate caused by heating the slightly acidified solution to 56° C.

Serum-globulin

This substance was formerly called fibrino-plastic substance by Schmidt,[3] and paraglobulin by Kühne.[4] The name serum-globulin was given to it by Weyl.[5] The serum-casein of Panum[6] has also been shown to be the same substance.

It may be prepared from blood plasma or exudation fluids (e.g. pericardial or hydrocele fluid) after they have been heated to 56° C. and the heat-coagulum of fibrinogen, which is formed at that temperature, removed by filtration. It is most frequently prepared from blood serum.

The following are the various methods that have been adopted for the preparation of serum-globulin:—

1. The serum is diluted with fifteen times its bulk of water, and a stream of carbonic acid passed through it. The precipitate is collected and washed with water that contains no oxygen dissolved in it (Schmidt).

2. The serum is similarly diluted, and a few drops of 2 per cent.

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[1] Herrmann, *Zeitsch. physiol. Chem.* xi. 508.

[2] Hammarsten, *Pflüger's Archiv*, xxii. 480.

[3] A. Schmidt, *Arch. f. Anat. u. Physiol.* 1861, p. 545, and 1862, p. 428.

[4] W. Kühne, *Lehrbuch d. physiol. Chem.* Leipzig, p. 174. This name is also used by Hammarsten, *Pflüger's Arch.* xvii. 413; xviii. 35.

[5] Weyl, *Zeitsch. f. physiol. Chem.* i. 77.

[6] Panum, *Archiv f. pathol. Anat.* iv.

acetic acid added. There is a small precipitate soluble in excess of the acid; a small precipitate is also produced by acetic acid after the removal of the precipitate produced by carbonic acid; hence it was supposed to be something different from serum-globulin and was called serum-casein (Panum). Hammarsten has, however, shown that both these methods produce but a very small precipitation of the globulin of serum, and that Panum's precipitate consists of the same substance as Schmidt's. There is no special serum-casein, or alkali-albumin in normal blood.

3. Mere dilution with ten to twenty times its volume of water will cause a small precipitation of the globulin from serum. In other words, globulins require a certain proportion of salts for their solution; this may be lessened by dilution, and hence the precipitation observed.

4. The same result is brought about by dialysing away the salts (see p. 120). The precipitation, however, is never complete.

5. Saturation with neutral salts. There are many salts that produce precipitation of the globulin.[1] Hammarsten was the first to point out that magnesium sulphate is the best to use for the purpose; it effects an absolutely complete precipitation of the globulin, and is for this reason preferable to sodium chloride,[2] which was employed originally by Denis, and subsequently by Schmidt. It precipitates none of the serum-albumin. Saturation with ammonium sulphate or potassium acetate precipitates all the proteids of the blood. It is stated by Kauder[3] that half-saturation with ammonium sulphate precipitates only the serum-globulin.

In all saturation experiments, the liquid should be approximately neutral to start with. Liquids like serum are, however, sufficiently near to the neutral point for the purpose, but in certain abnormal urines where globulin may be present, it is necessary to neutralise their acid reaction before saturation. In order to ensure complete saturation at the temperature of the air, it is necessary to thoroughly shake the mixture of salt and serum for two or three hours; this may be done most readily by a motor of some kind. The precipitate is collected on a filter and washed with a saturated solution of the salt used. When greater purity is required, the precipitate may be redissolved by adding distilled water; the water is able to dissolve the globulin by means of the salt adhering to it, and then it can be reprecipitated by saturation.

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[1] For a fuller account of the action of various salts *see* Halliburton, *Journ. of Physiol.* v. 176, *et seq.*; Lewith, *J. für experim. Path. u. Pharmakol.* xxiv. 1; Hofmeister, *Ibid.* p. 247.

[2] A full account of the solubilities of serum-globulin in solutions of sodium chloride of different strength will be found in *Pflüger's Archiv*, xviiii. 89 (Hammarsten).

[3] Kauder, *Arch. f. exp. Path. u. Pharmakol.* xx. 411.

In solutions containing at least 1 per cent. of globulin it may be detected by the ring of precipitate that occurs at the junction of the two liquids, when the solution is poured on to the surface of a saturated solution of magnesium sulphate.

Many of the distinctive characters of this globulin have necessarily been described in the foregoing paragraphs relating to its preparation. To these may be added the following :—

It coagulates on heating to 75° C., becoming opalescent a few degrees below that point. Its specific rotatory power for sodium light is 59·75° (Haas).

The estimation of serum-globulin quantitatively may be carried out as follows, in such liquids as serum. The serum is saturated with magnesium sulphate and filtered, the precipitate being collected on a filter of known ash which has been previously dried and weighed. The precipitate is washed with a saturated solution of magnesium sulphate, and then the filter with its adherent precipitate is dried at 120° C. This temperature in a few hours renders the globulin insoluble ; the salt is then washed away with water. It is subsequently washed with alcohol and ether, dried, weighed, incinerated, and the amount of ash deducted.

Estimations of serum-globulin made previous to Hammarsten's researches by means of the carbonic acid or dialysis methods are much too low and are practically valueless.

Sources of serum-globulin.—The total globulin in the serum is greater than that in the plasma, but the greater part is undoubtedly pre-existent in the plasma. Schmidt considered at one time that it was derived almost entirely from the disintegration of the white corpuscles, that occurs when the blood is shed. It is now generally admitted that a small, but a very small, quantity of the globulin is formed by such disintegration. This may be termed cell-globulin, and is closely allied to, or probably identical with, the fibrin-ferment. Lastly, some of the globulin is derived from the decomposition of the fibrinogen molecule when coagulation occurs (*see* p. 235). This second globulin of Hammarsten differs somewhat in its solubilities from the serum-globulin pre-existent in the plasma, and may be separated from it by fractional saturation with sodium chloride. The globulin of serum thus consists of a mixture of three globulins, all closely allied in their properties to one another ; these are :—

1. The globulin pre-existent in the blood plasma ; this may be termed *plasma-globulin.*

2. The globulin arising from the disintegration of the corpuscles—*cell-globulin.*

3. The globulin arising from the splitting of the fibrinogen molecule —*Hammarsten's second globulin.*

The last two are present in small and variable quantities; it is their presence that renders the elementary analysis of serum-globulin so difficult. The average percentage composition is as follows: C, 52·71; H, 7·01; N, 15·85; S, 1·11; O, 23·32. But these averages are taken from preparations in which the carbon varies from 53·3 to 52·32 (a difference of nearly 1 per cent.), and the nitrogen from 16·25 to 15·61 (a difference of 0·64 per cent.). Such differences cannot be regarded as coming within the limit of experimental errors, and in fact afford an additional proof that the globulin of serum has a different composition in different serums, or, in other words, is a mixture of two or more globulins (Hammarsten[1]).

The serum-globulin of serous exudations appears to be pure plasma-globulin, or at least it contains no cell-globulin.

The Fibrin-ferment

A. Buchanan[2] aptly compared the action of the white corpuscles in inducing coagulation to that of rennet in curdling milk. He thus anticipated to a certain extent the modern theory that the clotting of blood is due to the activity of a ferment. The fibrin-ferment, as it is called, was discovered by A. Schmidt,[3] and he prepared it in the following way from serum: the serum is mixed with ten to fifteen times its bulk of absolute alcohol; by this means the proteids are precipitated, as is also the ferment; the precipitate is left for six to eight weeks under the alcohol, by which time the proteids are rendered insoluble; the precipitate is collected, washed with absolute alcohol, dried in an exsiccator over sulphuric acid, and powdered. The ferment can be extracted by means of water from this powder. A solution of the ferment so prepared will cause the clotting of pericardial and similar coagulable fluids or of solutions of fibrinogen, and will hasten the coagulation of dilute salted plasma, or of pure plasma obtained by the experiments already described, such as the living test-tube experiment.

The circulating blood contains no ferment; if attempts be made to prepare it from blood received direct from the blood vessels of a living animal into absolute alcohol, the result will be a negative one.[4] The become a constituent part of the fibrin formed, though much of it remains adherent to the fibrin, as well as being dissolved in the serum.

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[1] Hammarsten, *Pflüger's Archiv*, xxii. 489, 490.

[2] Buchanan, *Lond. Med. Gazette*, xviii. 50.

[3] A. Schmidt, *Pflüger's Archiv*, vi. 413.

[4] This statement rests upon Schmidt's authority. Jakowicki (*Inaug. Dissert.* Dorpat, 1885) working under Schmidt's directions has, however, found a trace of ferment in living blood, but so small and so inactive that Schmidt's original statement may still be considered as practically true.

There are other globulins which have the same effect upon the formation of fibrin that cell-globulin has. For instance, the myosinogen of muscular tissue is one of these (*see* Muscle).

The addition of living cells, such as yeast cells, or pieces of many fresh tissues, to such liquids as hydrocele fluid or dilute salted plasma causes a rapid formation of a clot. In these cases, if neither cell-globulin nor myosinogen is present, in all probability there are similar unstable globulins in the cell-protoplasm which act in the same way.

Historical account of the theories of Coagulation

Nearly up to the end of the eighteenth century the clot was supposed to consist of merely adherent corpuscles. This view was held in Britain by Keill, Jurin, Thomas Morgan, John Cook, Arbuthnot, Cowper, Langrish, Berdoe, and others; and on the Continent by Leeuwenhoek, Boerhaave, Van Swieten, Haller, and Marherr. Petit, Quesnay, Senac, Borelli, and Davies were the earliest to have an idea of a coagulable substance in addition to the cells, and this was fully recognised and proved by Hewson (1772), and taught by Fordyce and the Hunters.[1]

The reason that the blood coagulates outside the vessels and not during life was accounted for in different ways; some considered that coagulation was due to the action of the air on the blood (Borelli, Lower); others that the blood was maintained in its liquid condition during life by its continual movement; others, again, that coagulation was due to the cooling of the blood on its removal from the vessels.[2] We however now know, on the contrary, that blood will clot even if collected over mercury without coming in contact with the air at all, that agitation hastens and cooling hinders coagulation. Hunter considered that coagulation was an act of life, and connected with the vitality of the blood—a vague statement which implies very little; but, as Gulliver[3] pointed out, if it is a vital act, it is equivalent to saying that we are able to pickle the life of the blood for hours or even days, although decomposition may have begun in other parts of the body.

Hewson not only showed that a coagulable substance we now call fibrin separates from the plasma, which he obtained by skimming it off from the surface of blood which coagulated slowly, but he also discovered the fact that cold, contact with living vessels, and admixture with salts are agencies which hinder or prevent coagulation. In connection with the influence of the living vessels on coagulation, the further researches of Lister, Fredericq, and Brücke have been already referred to (*see* p. 224).

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[1] For the references to the writings of these authors see *Hewson's Works*, edited by Gulliver, *Sydenham Soc.* p. xxix. *et seq.*

[2] For a *résumé* of these earlier views, I am indebted to Gamgee, *Physiol. Chem.* p. 42.

[3] *Hewson's Works*, note 12, p. 21.

Andrew Buchanan[1] was the next who made noteworthy investigations into this subject. He experimented with fluid obtained from the pericardial sac and from the tunica vaginalis in the dropsical condition of that serous membrane called hydrocele. These liquids do not coagulate spontaneously, but Buchanan found that the addition of small shreds of 'washed blood clot' caused the formation of fibrin in them. This power was exhibited to a greater extent still by the 'buffy coat' of a clot; he therefore concluded that the power resided in the white blood corpuscles which are so abundant in the buffy coat, and their action he compared to the action of rennet in curdling milk.

Then came Denis,[2] who saturated blood plasma with sodium chloride, and thus obtained a proteid precipitate. This precipitate was washed with a saturated solution of sodium chloride and redissolved by the addition of water, the adherent salt rendering it soluble. This solution remained liquid for a short time, but on being allowed to stand a clot of fibrin was produced. Denis had thus obtained the precursor of fibrin from the plasma, and to it he gave the name *plasmine*; the proteids, which were not precipitated by the salt, he called *serine*, or, as we now call them, serum-albumin. We now know that Denis' plasmine was a mixture of fibrinogen, serum-globulin, and fibrin-ferment.

Alex. Schmidt[3] recognised these three substances; he, however, supposed that all three were necessary for the formation of a clot. One of the most important experiments on which he based this view was, that if serum which contains serum-globulin (or fibrino-plastic substance, as he termed it) and ferment be added to hydrocele or pericardial fluid, which he supposed contained fibrinogen but no serum-globulin, the result is the formation of fibrin. He also found that the more serum-globulin he added to a coagulable liquid the larger was the yield of fibrin from it.[4]

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[1] A. Buchanan, *London Med. Gazette*, xviii. (1835), p. 50; also vol. i. new series (1845), p. 617. The latter paper was reprinted by Dr. Gamgee in the *Journ. of Physiol.* vol. ii. (1879). The influence of leucocytes in bringing about coagulation was very strongly insisted on by Mantegazza (*Centr. Med. Wiss.* 1871, p. 709).

[2] Denis, *Mémoire sur le sang*, 1859, p. 32.

[3] A. Schmidt, *Arch. f. Anat. u. Physiol.* 1861, p. 545; 1862, pp. 428 and 533. *Pflüger's Archiv*, vi. 445.

[4] In connection with the question whether or not the ferment is a globulin, it is interesting to note that the proteid present in Schmidt's ferment solutions, and which some have considered as an impurity, was one which was precipitable by a stream of carbonic acid; he also found that serum *minus* its globulin has very little ferment activity; that it still possesses any is due to the fact that Schmidt's carbonic acid method does not completely precipitate the globulin.

O. Hammarsten[1] ascertained the characters of fibrinogen, serum-globulin, and fibrin-ferment with greater exactness, and showed that serum-globulin, or paraglobulin, as he terms it, is not necessary for the formation of fibrin, but that fibrinogen is the only precursor in the plasma of the fibrin in the clot. He pointed out that pericardial and hydrocele fluids contain abundance of serum-globulin, as well as fibrinogen, and therefore the addition of serum causes these fluids to coagulate, not in virtue of the serum-globulin, but of the ferment it contains. He pointed out that serum-globulin is very difficult to separate from ferment, a fact which is easy to understand, as we now know that the ferment is probably itself a globulin; the addition of apparently pure serum-globulin, prepared from serum, to hydrocele fluid causes the formation of fibrin because of its admixture with ferment. A pure serum-globulin prepared from pericardial or hydrocele fluid has on the other hand no fibrinoplastic activity. The most striking proof, however, of Hammarsten's theory is this: that a solution of the ferment added to a solution of pure fibrinogen causes the formation of fibrin.

It should be mentioned that Hammarsten does not regard the ferment as a globulin, because he is able to prepare it from horse's serum which has apparently been deprived of all globulin by saturation with magnesium sulphate. Howells[2] and also Hayem[3] have tried this method with the blood of other animals, but unsuccessfully, and in the course of my own work I have done the same, and again with a negative result. In the case of horse's serum, however, I have found that Hammarsten's statement is correct; and the explanation seems to be that for some reason or other it is exceedingly difficult to precipitate all the globulin from horse's blood by the use of this salt; but after repeated saturations one can remove all the globulin, and with it all ferment activity also.

The researches of Gamgee, of Lea and Green, and of myself, into the nature of the fibrin ferment have been already alluded to (*see* p. 241). To Green also we owe the discovery that the presence of calcium sulphate is necessary for the proper action of the ferment to take place. This again reminds us of Buchanan's old comparison of the clotting of blood to the curdling of milk, where the phosphate of calcium is a *sine qua non*.

Although Hammarsten did not consider serum-globulin necessary for the formation of fibrin, he admitted that its presence was advantageous; it can, however, be replaced by other proteids like casein, or even by salts like calcium chloride. He considers that serum-globulin possibly acts like calcium chloride in combining with the alkaline carbonates present in the blood, the presence of which would otherwise impede the activity of the ferment. The supposed action of calcium chloride in this respect may be represented by the formula

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[1] O. Hammarsten, *Pflüger's Archiv*, xiv. 211; xvii. 413; xviii. 38; xix. 563; xxii. 489.

[2] Howells, *Studies from the Physiol. Lab. Johns Hopkins Univ. Baltimore*, vol. ii.

[3] *Du sang*, Paris, 1889.

$$Na_2CO_3 + CaCl_2 = 2NaCl + CaCO_3.$$

Of recent years an entirely new theory was advanced by the late Dr. Wooldridge, which may be stated as follows:[1]—The coagulation of the blood is a phenomenon essentially similar to crystallisation; in the plasma there are three constituents concerned in coagulation, A, B, and C fibrinogen. A and B fibrinogen are compounds of lecithin and proteid, and fibrin results from the transference of the lecithin from A-fibrinogen to B-fibrinogen. C-fibrinogen is what has hitherto been called fibrinogen; A-fibrinogen is a substance which may be precipitated by cooling 'peptone plasma,' and on the removal of this substance coagulation occurs with great difficulty. The precipitate produced by cold consists of rounded bodies resembling the blood tablets in appearance. He further found that other compounds of lecithin and proteid, to which he has extended the name of fibrinogen, exist in the testis, thymus, and other organs, in the fluid of lymph glands, in the stromata of red corpuscles, and in the serum of certain animals: these substances may be extracted from the organs by water, and precipitated from the aqueous extract by acetic acid, and on redissolving this in a saline solution, and injecting it into the circulation of a living animal, intravascular clotting occurs, which results in the death of the animal. The form of fibrinogen that acts thus, he looks upon as the precursor of A-fibrinogen. From these points of view the fibrin-ferment and the white corpuscles are looked upon as of secondary import in causing coagulation, though it is admitted that fibrin-ferment converts C-fibrinogen into fibrin.

I have elsewhere[2] given at some length my reasons for not accepting this theory, and this is not the place for debating a controversial subject. I will merely say that I still consider Wooldridge erred, first, in drawing conclusions from observations on peptone plasma without corroborating them by experiments on pure plasma; and, secondly, in attributing to the corpuscular elements a secondary rôle in the causation of clotting.[3]

The latest theory of coagulation is that of Freund;[4] he considers that, when blood is shed, earthy phosphates derived chiefly from the corpuscles unite with fibrinogen and thus form fibrin.

Serum-Albumin

Serum-albumin, or serine, is the proteid which remains in solution after the separation of serum-globulin from the serum. Now that Hammarsten's method has been adopted for the separation of serum-globulin, we know that it is more abundant than had been previously supposed, and may in certain serums be present in even greater abundance than serum-albumin.

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[1] Wooldridge, *Ludwig's Festschrift*, 1886, p. 221.

[2] *Journ. of Physiol.* ix. 270. Wooldridge defended his views in the same Journal, x. 339.

[3] Krüger (*Zeit. Biol.* xxiv. 189) and Hayem (*Du sang*, Paris, 1889), who have also recently examined Wooldridge's views, are unanimous in regarding the corpuscles as most important factors in fibrin-formation.

[4] *Med. Jahrb.* 1888, p. 259.

Serum-albumin may be prepared from serum which has been freed by Hammarsten's process from serum-globulin in the following way: The precipitated globulin is filtered off; the filtrate, which is already saturated with magnesium sulphate, is then saturated with sodium sulphate;[1] the result is a precipitation of serum-albumin in the form of fine flocculi; this is washed with water saturated with the two salts, and is readily soluble on the addition of water.

If sodium sulphate is added to serum which has not been treated with magnesium sulphate, there is little or no precipitate. Magnesium sulphate alone precipitates the globulin; double saturation with the two salts precipitates both the proteids of the serum. The explanation of this is that when sodium sulphate is added to magnesium sulphate, the double sulphate of magnesium and sodium is formed, and this it is which precipitates the serum-albumin. The formula for magnesium sulphate is $MgSO_4.7H_2O$; that of sodio-magnesium sulphate is $MgSO_4.Na_2SO_4.6H_2O$; that is, a molecule of sodium sulphate takes the place of one of the molecules of water of crystallisation of the magnesium sulphate.

Sodio-magnesium sulphate is obtained commercially as a by-product in the manufacture of Epsom salts. Saturation of solutions of proteids, such as serum, with this salt causes a complete precipitation of all the proteids contained therein. Like ammonium sulphate it does not precipitate peptones.[2]

This method affords us a means by which we may obtain serum-albumin free from other proteids; it may be further purified by reprecipitating it from the solution with the double sulphate, and if necessary it may be again dissolved and reprecipitated. The adherent salt may be ultimately removed by dialysis.

It is, however, not possible to completely remove the salts from this or any other proteid. A small quantity of ash (0·3 to 0·5 per cent.) will always be obtained from the purest preparations.

Aronstein and Schmidt stated that they obtained serum-albumin free from ash by means of dialysis, and that this pure product did not coagulate on heating. No one has been able to confirm these experiments, and the non-coagulation noted by Aronstein and Schmidt was in all probability due to the adherence of alkaline salts in small quantities to the proteid.[1]

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[1] Sodium nitrate, ammonia alum, and potassium iodide act similarly.

[2] The action of sodium sulphate in precipitating serum-albumin has been worked out by Denis (*Mémoire sur le sang*, p. 39), Schäfer (*Journ. of Physiol.* iii. 181), and Halliburton, *Ibid.* v. 177 *et seq.*

Serum-albumin gives the usual proteïd tests; it differs from globulins in its solubility in water, and the fact that it is less readily precipitated by saturation with neutral salts. It differs from egg-albumin in its specific rotatory power:[2]

Egg-albumin: Specific rotation for yellow light $(\alpha)_D = -33{\cdot}5°$.

Serum-albumin: Specific rotation for yellow light $(\alpha)_D = -56°$.

Ether does not precipitate serum-albumin: it does precipitate egg-albumin.

Further investigation has shown that serum-albumin is not a single substance, but probably a mixture of several albumins. By fractional heat-coagulation it is possible to separate it into three proteids, coagulating α at 73°, β at 77°, and γ at 84°C.[3] (*see* p. 118). The results of elementary analysis also lead to the same conclusion.[4]

The following tables illustrate certain points in the comparative chemistry of serum and plasma.

TABLE I

Animal	Proteids in the Blood-Serum[5]		
	Total Proteids per cent.	Serum-Globulin	Serum-Albumin
Man[6]	7·62	3·10	4·52
Horse[6]	7·25	4·56	2·67
Ox[6]	7·50	4·17	3·33
Rabbit[6]	7·52	1·78	4·43
Pigeon[7]	5·01	1·32	3·69
Hen[7]	4·14	2·90	1·24
Tortoise[7]	4·76	2·82	1·94
Lizard[7]	5·16	3·33	1·83
Terrapin[9]	5·25	4·66	0·69
Snake[10]	5·32	4·95	0·37
Frog[8]	2·54	2·18	0·36
Newt[7]	3·74	3·31	0·43
Eel[7]	6·73	5·28	1·45
Dogfish[7]	1·62	1·17	0·45

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[1] Aronstein, *Pflüger's Archiv*, viii. 178. A. Schmidt, *Ludwig's Festgabe*, 1874, p. 94; *Pflüger's Archiv*, ii. 1. Heynsius, *Ibid.* ix. 514. Winogradoff, *Ibid.* ii. p. 605. Huizinga, *Ibid.* ii. 392. Haas, *Ibid.* xii. 378–410.

[2] Hoppe-Seyler, *Zeitsch. f. Chem. u. Pharm.* 1864, p. 737.

[3] Halliburton, *Journ. of Physiol.* v. 152.

[4] Kauder, *Arch. f. exp. Path. u. Pharmak.* xx. 411.

[5] The total proteids are estimated by weighing the precipitate produced by adding alcohol to the serum; the globulin is estimated in another portion by Hammarsten's method (p. 288); the difference between the two gives the amount of albumin.

[6] Hammarsten, 'Ueber das Paraglobulin,' *Pflüger's Archiv*, 1878.

[7] Halliburton, *Journ. of Physiol.* vii. 321. [8] May, *Ibid.* p. 319.

[9] Howells, *Studies from the Biol. Lab. Johns Hopkins Univ. Baltimore*, iii. 49.

[10] Wolfenden, *Journ. of Physiology*, vii. 323.

TABLE II[1]

Blood of	Heat-Coagulation Temperature of				
	Fibrinogen	Serum-Globulin	Serum-Albumin		
			α	β	γ
Man	56° C.	75° C.	73° C.	77° C.	85° C.
Monkey . . .	56°	75°	72°	77°	83°
Dog	56°	75°	73°	78°	84°
Cat	56°	75°	73°	77°	84°
Rabbit . . .	56°	75°	73°	77°	84°
Pig	56°	75°	72°	77°	84°
Horse . . .	56°	75°	—	77°	84°
Ox	56°	75°	—	77°	84°
Sheep . . .	56°	75°	—	77°	84°
Hen	56°	75°	72–3°	78°	86°
Dove	56°	75°	73°	77°	85°
Newt . . .	56°	75°	73°	—	—
Toad	56°	75°	75°		—
Frog	56°	75°	73°	—	—
Lizard . . .	56°	75°	74°	—	-
Perch . . .	56°	75°	73°	—	—
Roach . . .	56°	75°	73°	—	--

The above tables give merely illustrative examples from the different groups of the vertebrate kingdom. From them the following conclusions can be drawn :—

1. The temperature of heat-coagulation of the two globulins of the plasma (fibrinogen and serum-globulin) is exceedingly uniform throughout the vertebrate kingdom. The small amount of fibrinogen, as judged by the smallness of the clot in cold-blooded animals, has been already alluded to (p. 222).

2. In warm-blooded animals, mammals and birds, the serum-albumin can be differentiated into three proteids by a process of fractional heat-coagulation. In certain ungulates, however (horse, ox, sheep), only two varieties (β and γ) of serum-albumin are present.

3. In cold-blooded animals, the proteids differ from those of warm-blooded animals in the following points :-

(*a*) The percentage of total proteids is smaller.

(*b*) The serum-albumin is especially diminished, not only absolutely but relatively to the serum-globulin present.

(*c*) The serum albumin is a single proteid, corresponding to that called serum-albumin *α* in the higher vertebrates.

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[1] Compiled from Papers by myself in the *Journ. of Physiol.* v. 159, and vii. 320; *Quart. Journ. of Mic. Science*, xxviii. 198.

It has been stated that during starvation the serum-albumin diminishes more quickly than the serum-globulin; in the case of snakes, Tiegel has said that the albumin altogether disappears. Burckhardt stated that much the same occurs in dogs. These observers did not, however, employ the only exact means of estimating the proportion of globulin and albumin, which is Hammarsten's magnesium sulphate method. Howells and Salvioli, who employed this method, showed that the observations of Tiegel and Burckhardt were incorrect.[1]

In concluding the subject of the proteids of the plasma and serum, it may be added that no proteoses (albumoses) or peptones are to be found in normal blood, even in the portal circulation when absorption is taking place. This subject will be discussed at greater length in the chapter on Absorption.

The following scheme represents a method of separating the proteids of the plasma or of similar fluids :

Plasma. Add an equal volume of saturated solution of sodium chloride. A precipitate is produced.

Filter.

Precipitate : consists of FIBRINOGEN	Filtrate contains the remaining proteids. Saturate with magnesium sulphate ; a precipitate is produced. Filter.
Precipitate : consists of SERUM-GLOBULIN	Filtrate contains SERUM-ALBUMIN. Heat to 73°C. ; a precipitate is produced. Filter.
Precipitate is a heat-coagulum of serum-albumin α	Filtrate contains serum-albumin β and γ. Heat to 77° C. ; a precipitate is produced. Filter.
Precipitate is a heat-coagulum of serum-albumin β	Filtrate contains serum-albumin γ, which is precipitated at 84° C.

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[1] For reference to this subject see *Journal of Physiology*, vii. 322.

The following is another method:—

Allow either spontaneous coagulation to take place, or in the case of salted plasma, dilute and add fibrin-ferment. A clot forms, and a liquid residue called serum can be filtered off.

Clot=fibrin

Liquid residue=serum. Saturate with magnesium sulphate ; a precipitate forms. Filter.

Precipitate=SERUM-GLOBULIN

Filtrate contains SERUM-ALBUMIN.

The following method illustrates how by the use of various salts the proteids may be separated.

PLASMA.—Saturate with ammonium sulphate ; a precipitate forms. Filter.

Precipitate consists of PROTEIDS. Wash with saturated solution of ammonium sulphate. Redissolve the precipitate by the addition of water. All the proteids dissolve. Saturate this solution with magnesium sulphate ; a precipitate forms. Filter.

Filtrate contains the other constituents of the plasma.

Precipitate = GLOBULINS (plasmine — Denis) (=fibrinogen + serum-globulin). Wash this precipitate with saturated solution of magnesium sulphate ; then add water, the globulins redissolve owing to the presence of adherent salt. Heat this solution to 56°C. ; the fibrinogen is precipitated as a heat-coagulum at 56°, the serum-globulin remaining in solution. *Or* add an equal volume of saturated solution of NaCl ; a pp. forms. Filter.

{ Precipitate=fibrinogen
{ Filtrate contains serum globulin.

Filtrate contains ALBUMINS which may be precipitated and separated by fractional heat-coagulation. *Or* saturate the solution with sodium sulphate ; a precipitate forms. This precipitate = albumins, which may be redissolved in water, and then separated by heat-coagulation.

EXTRACTIVES OF THE PLASMA AND SERUM

We now come to the second group of organic substances in the liquor sanguinis. They are called extractives, because they can be extracted from the various liquids of the body by reagents like alcohol and ether; they are present only in small quantities. The extractives of the serum are the same as those of the plasma.

Ether extractives.—These consist of neutral *fats*, and *cholesterin*, and in quantity vary from 0·2 to 0·6 per cent. The fats are more abundant after a fatty meal; and minute fat globules can then be recognised in the serum by the microscope. Sometimes this is so marked that the serum looks milky like the chyle. The fats present are the same as those in adipose tissue (palmitin, stearin, olein). Röhrig[1] stated that soluble soaps cannot exist in the blood, as was at one time supposed; but Hoppe-Seyler[2] has pointed out errors in Röhrig's analyses, and found from 0·05–0·1 per cent. of soaps in serum. About one-tenth of the ethereal extract consists of cholesterin,[3] the chemistry of which will be taken with the nervous tissues, and bile. *Lecithin* is also present in small quantities. The chemistry of this substance will be treated of in connection with nervous tissues.

Nitrogenous compounds.—Urea and uric acid are found in small quantities in the blood; the quantity of urea varying between 0·02 and 0·04 per cent. in human blood.[4] Creatine, xanthine, hypoxanthine, and hippuric acid are found in still smaller quantities. In certain diseases they are increased in quantity, and other substances belonging to the same category may appear, e.g. leucine and tyrosine in acute yellow atrophy of the liver.

In certain forms of Bright's disease the amount of urea is much increased; and in gout and allied affections there is an increase of the uric acid. It will be convenient here to mention the way in which these two substances may be estimated.

Estimation of urea in blood or serous liquids.—Haycraft's[5] method is by far the best, and may be described as follows: The blood is placed in a parchment-paper dialyser, and outside the dialyser is a vessel containing absolute alcohol. The urea passes out in a few hours into the alcohol, leaving a solid coagulum of corpuscles and proteids inside the dialyser.

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[1] Röhrig, *Ludwig's Arbeiten*, 1874.

[2] Hoppe-Seyler, *Zeit. physiol. Chem.* viii. 503.

[3] Hoppe-Seyler, *Med. Chem. Untersuchungen*, p. 145.

[4] Gamgee, *Physiol. Chem.* p. 65.

[5] Communicated privately to Dr. Gamgee, and published by him in his *Physiol. Chemistry*, p. 192.

To this a little water is added, and dialysis is allowed to take place into a fresh supply of alcohol. This may be again repeated. The alcohol is then acidified with oxalic acid, and evaporated to dryness. The fat and pigment are removed from the residue by petroleum naphtha, the oxalate of urea remaining undissolved; this is dissolved in water, neutralised with barium carbonate, evaporated to dryness, and the urea is extracted from the residue by boiling alcohol. On evaporating off the alcohol, pure urea is left which may then be weighed, or a quantitative determination made by the hypobromite method (*see* Urine).

Estimation of uric acid in blood or serous liquids.—The following is Garrod's method.[1] The serum of the blood or a fluid of a blister is dried, powdered, extracted with hot alcohol, and the residue digested with boiling water. The watery solution is filtered and concentrated; it gives the murexide test, and after the addition of acetic acid, crystals of uric acid form which can be collected and weighed.

A very good clinical method in cases of gout, consists in placing a linen fibre in the concentrated serum to which acetic acid has been added. The crystals collect on the thread, and these can be recognised under the microscope, or by the murexide test.

Sugar.—Dextrose is present in small quantities in the blood, but most abundantly after a starchy meal in the portal circulation. The normal quantity in dog's blood varies from 0·1 to 1·2 per 1000 (Pavy,[2] v. Mering[3]). Seegen[4] has shown that the form of sugar in the blood is dextrose—not maltose, as some have supposed.

Seegen[5] states the quantity of sugar in the blood leaving the liver, that is, in the hepatic vein, is always greater than that in the blood going to the liver either by the hepatic artery or by the portal vein. He gives the following average results in the dog from a large number of experiments:—

Normal amount of sugar in	cardiac and arterial blood	0·1–0·15	per cent.
,,	portal blood	0·119	,,
,,	hepatic blood	0·23	,,

The following four experiments performed on non-anæsthetised animals (chloroform and ether narcosis vitiating the results somewhat) illustrate the same point.[6]

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[1] Garrod, Art. 'Gout,' *Reynolds's System of Medicine*, vol. i. p. 825; *Med. Chir. Trans.* xxxvii. p. 826.

[2] Pavy, *Croonian Lectures*, 1878.

[3] v. Mering, *Archiv f. Anat. u. Physiol.* 1877, p. 380.

[4] Seegen, *Pflüger's Archiv*, xl. 38–64.

[5] Seegen, *Bied. Centralbl.* 1884, p. 747.

[6] Seegen, *Pflüger's Archiv*, xli. 526.

	Percentage of sugar	
	Portal blood	Hepatic blood
1.	0·101	0·258
2.	0·090	0·175
3.	0·107	0·209
4.	0·120	0·287

This subject will be fully considered in connection with the functions of the liver, and absorption, and also in connection with the disease called diabetes, in which there is a great excess of dextrose in the blood, much of which passes into the urine. It will be, however, convenient to describe here the methods of estimating sugar in the blood. One must first get rid of the proteids. Pavy precipitates these by adding sulphate of soda and boiling. v. Mering merely dilutes the serum with four or five times its volume of water, boils, and then adds a little dilute acetic acid; the heat-coagulum which forms is then filtered off, washed, and the washings added to the filtrate. In the filtrate in either case the sugar is determined by means of Fehling's solution (*see* p. 98).

Pigment.—The serum is differently tinted in different animals: thus the ox, sheep, and man have serum with a yellow tinge; in birds it is a deeper orange; in the monkey, dog, cat, rabbit, and in most cold-blooded animals the serum has either a faint colour, or is almost colourless.

Hammarsten,[1] MacMunn[2] and others have described this pigment as identical with that of bile; and there is no doubt that this may be the case in certain animals, or more especially in certain diseases which are accompanied by the condition known as jaundice.

The normal pigment of the serum and plasma seems, however, to belong to the class of colouring matters known as lipochromes or fatty colours. One of the first described of these was found in the corpus luteum of the ovary, and the name lutein was given to it by Thudichum,[3] and has since been extended to the whole group. Krukenberg's word lipochrome seems, however, to designate the class of pigments more correctly. They are soluble in the reagents in which fats are soluble (ether, alcohol, turpentine, &c.); they give certain characteristic colour reactions (e.g. a blue colour with iodine and sulphuric acid); they exhibit absorption spectra in which the bands are situated towards the violet end of the spectrum, and they are bleached by light (*see* p. 148).

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[1] Hammarsten, *Maly's Jahresbericht*, 1878, p. 129.

[2] MacMunn, *Proc. Roy. Soc.* xxxi. 231.

[3] Thudichum, *Centralbl. f. d. med. Wissensch.* vii. 1879, p. 1.

Krukenberg[1] has extracted such a lipochrome or serum-lutein from ox blood by means of amyl alcohol; but I have found[2] that in the case of birds' and turtles' blood, and more recently in the case of mammalian blood also, ordinary ethylic alcohol or ether will do equally well.

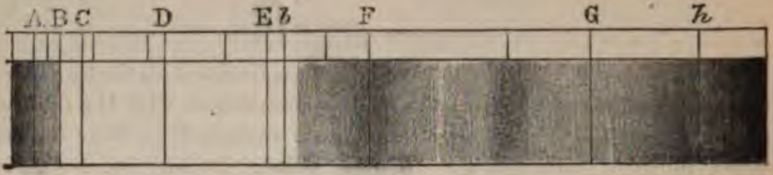

FIG. 53.—Absorption spectrum of Serum-lutein. There is a large absorption of the violet end of the spectrum, and in this two darker shadows are to be made out, which are the absorption bands mentioned in the text.

Serum-lutein shows two ill-defined absorption bands, one in the region of the F line, and one between the F and G lines. It differs from the lipochromes, which we shall come across in the retina and elsewhere, in being insoluble in turpentine.

THE INORGANIC CONSTITUENTS OF THE PLASMA AND SERUM

If all the organic material (proteids and extractives) in the dry residue of either the plasma or the serum be burnt away, a mineral ash remains. That this was pre-existent in the liquid under investigation, and not simply produced by the ignition of the organic substance, is shown by the fact that many of the salts can be identified in the plasma or serum itself, e.g. on concentration crystals of sodium chloride form. The method of investigating the mineral constituents of the blood in the unaltered serum has been chiefly adopted by Pribram and Gerlach,[3] who have shown that many previous observations were incorrect or inaccurate, which were based simply upon examination of the ash left after ignition.

The salts of the blood, like the extractives, pass into the serum when clotting occurs. A certain small quantity is however held by the fibrin; and as in the case of serum-albumin, it is not possible even after the most prolonged dialysis to separate all of this salt from the fibrin. The serum thus contains a slightly smaller percentage of salts than the plasma.

As will be seen by consulting the following tables, sodium chloride is by far the most abundant salt present; it constitutes between 60 and 90 per cent. of the total ash. Potassium chloride is present in much smaller amount. It constitutes about 4 per cent. of the total ash.

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[1] Krukenberg, *Sitzungsb. d. jenaischen Gesellsch. f. Med.* 1885.

[2] Halliburton, *Journ. of Physiol.* vii. 324.

[3] *Ludwig's Arbeiten*, 1871 and 1872.

Sodium carbonate (Na_2CO_3) is, next to the chloride, the most plentiful ingredient of the ash left after ignition. In the plasma and serum, however, the carbonate present is probably sodium hydrogen carbonate or bicarbonate of soda, $NaHCO_3$.

Lastly there are phosphates of calcium, magnesium, and sodium, and a small amount of sulphate of potassium. It is, however, uncertain whether the phosphates are metaphosphates or orthophosphates. The researches of Pribram and Gerlach have shown that the phosphates in the ash are greater in amount than those actually existing in the serum; this is because a certain quantity of phosphoric acid is formed from lecithin and similar organic compounds of the serum which contain phosphorus, during the process of incineration.

Salts of the plasma (C. Schmidt). 1000 parts of plasma yield:—

Mineral matter	8·550
Chlorine	3·640
Sulphuric anhydride (SO_3)	0·115
Phosphoric anhydride (P_2O_5)	0·191
Potassium	0·323
Sodium	3·341
Calcium phosphate	0·311
Magnesium phosphate	0·222

Phosphoric anhydride and calcium in serum (Pribram). 1000 grams of serum yield:—

Phosphoric anhydride (P_2O_5)	0·179
Calcium oxide (CaO)	0·173

Magnesium in serum (Gerlach). 1000 grams of serum yielded:—

(1) Magnesium oxide (MgO)	0·025
(2) " " "	0·027

Method of Investigation.—The organic matters of the solid residue of serum or plasma may be simply burnt off in a crucible, and the ash examined according to the methods of inorganic analysis for acids and bases. The proportions in which these occur give data for calculating the probable manner in which they were united as salts in the fluid under investigation. This method is, however, far from accurate, as certain volatile constituents of the ash, such as sodium chloride, are partially lost: and in the case of the phosphates we have seen that other causes are at work to produce inaccuracy.

Rose's method consists in heating the dry residue very cautiously until it is carbonised. The contents of the crucible are then extracted with hot water again and again to remove all soluble salts. The insoluble residue is then ignited, and the weight of the residue gives the amount of the constituents of the ash which are insoluble in water. The weight of those which are soluble in water can be then ascertained by evaporating the watery extracts to dryness, and igniting the solid residue at a red heat.

It will be found that the following method of destroying organic matter is far better than incineration: evaporate to dryness, and then heat with fuming nitric acid for some hours in a water-bath, adding more acid from time to time till all effervescence ceases. Finally evaporate to dryness: the saline matters only are present in the residue.

Pribram and Gerlach have shown that calcium, magnesium, phosphoric acid, and sulphuric acid may be precipitated from serum by the same methods as in aqueous solutions. The precipitates are collected by centrifugalising, and then washed by decantation. This method of direct precipitation also avoids all those sources of error that result from the process of ignition.

The physiological importance of the inorganic constituents of the blood.—It is well known that distilled water acts like a poison to protoplasm; fishes kept in it die quickly, cilia stop moving, white blood corpuscles burst. Thus in physiological research one commonly uses a 0·6 per cent. solution of sodium chloride in order to keep muscles, nerves, &c., moist during an experiment. Dr. Ringer[1] has shown, by a large number of observations on fish, tadpoles, cilia, skeletal muscle, but more especially in connection with the heart, the relative importance and action of the different salts of the blood. Minute quantities of such salts, such as occur, for instance, in river water, are quite sufficient to keep fish alive for weeks, which would die in distilled water in a few hours. Kronecker[2] and his pupils maintain that the frog's heart does not feed on its own substance, but that as soon as nutritive fluid is withdrawn its contractions stop. They state that fluids only, which contain serum-albumin will sustain the heart's contractility. They showed that a 0·6 per cent. solution of sodium chloride soon stops the heart, and so it undoubtedly does. Merunowicz,[3] however, finds that the dissolved ash of incinerated blood supports the heart's contractility, and Ringer has shown that a good circulating fluid for the heart may be compounded by mixing small quantities of such salts as nominally occur in the blood, and that with this fluid the heart after removal will continue to beat normally as long, or nearly as long, as it does with defibrinated blood. The necessity for lime salts is especially great; in fact the close adhesion of proteids generally with small quantities of mineral matter is rather suggestive of combination than mere mixture. Lime salts adhere especially closely, and, in fact, seem indispensable for many of the functions of the body, of which the beating of the heart and the contraction of skeletal muscle are good examples. Blood from which the salts have been removed by dialysis keeps the heart going, but the tracing is abnormal, resembling that produced by a weak solution of a lime salt; it is in fact found that dialysis will not remove the lime from serum-albumin, though it removes the greater part of the sodium and potassium salts. Saline solutions like Ringer's circulating fluid contain salts of all three metals; they have been employed for transfusing into the blood vessels of persons who have suffered severely from hæmorrhage.

The following is the composition of Ringer's circulating fluid:—

100 c.c. of a 0·75 per cent. solution of sodium chloride.
1 c.c. of a 1·0 per cent. solution of calcium chloride.
1 c.c. of a 0·75 per cent. solution of potassium chloride.
1 c.c. of a 1·0 per cent. solution of bicarbonate of soda.

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[1] S. Ringer, *Journ. of Physiol.* iii. 380; iv. 29, 222; v. 98; vi. 361; vii. 118, 291; viii. 15, 20, 288; xi. 79.

[2] Martius, *Du Bois Reymond's Archiv*, 1881, p. 474. Kronecker and Von Ott, *Ibid.* p. 569. Martius, *Ibid.* 1882.

[3] *Arbeiten aus der physiologischen Anstalt zu Leipzig.*

The following has, however, been more recently proved by Ringer to act even better than the above :—

100 c.c. of a 0·75 per cent. solution of sodium chloride
saturated with calcium phosphate
1 c.c. of a 2·0 per cent. solution of potassium chloride.

Ludwig's circulating fluid contains a trace of commercial peptone in addition to inorganic constituents. Its composition is as follows:—

100 c.c. of water
0·5 gram of sodium chloride
0·002 gr. of potassium hydrate
0·003 gr. of peptone.

(The commercial peptone contains the necessary lime salts.)

THE WHITE BLOOD CORPUSCLES OR LEUCOCYTES

The white blood corpuscles are typical animal cells ; they consist of more or less granular masses of protoplasm, containing a nucleus in the centre. Their protoplasm exhibits movements, which are termed amœboid, from their resemblance to the movements of the amœba. Amœboid movement was first observed in white blood corpuscles by Wharton Jones.[1] These movements can be most readily observed with the microscope, while using a warm stage the temperature of which is about 40°C. It is by virtue of such movements that locomotion becomes possible to these corpuscles; this may lead to their emigration from the blood vessels, and when in the tissues, they are termed wander-cells. Emigration takes place to a much greater extent than normal in inflamed parts, and may go to such an extent as to cause the formation of an abscess, i.e. a collection of pus or white blood corpuscles suspended in a fluid like serum in composition.

It is in virtue of their amœboid movements, and power of assimilation, that white blood corpuscles are enabled to take up nutritive substances from the lining membrane of the alimentary canal. Fat globules for instance can be readily seen in these corpuscles at a certain stage during absorption.

White blood corpuscles also disintegrate readily ; the changes which occur in them when the blood is shed have been already referred to (p. 240). They also probably disintegrate in the lacteals or lymphatic vessels of the intestine ; and in so doing, liberate the nutritious substances derived from the alimentary canal.

White blood corpuscles are not nearly so numerous in the blood as are the red. Their number varies with age, sex, period after food and region from which the specimen of the blood is taken. On an average in man there is one white corpuscle to every 350 red ones, i.e.

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[1] Wharton Jones, *Phil. Trans.* 1846.

about 15,000 in every cubic millimetre of blood. They are not constant in size, but in man they average about 0·01 millimetre ($\frac{1}{2500}$ inch) in diameter; they are somewhat larger in the lower vertebrate groups. They are always found in greater abundance on the upper surface of a blood clot than in the lower part, a fact which shows that their specific gravity is less than that of the red corpuscles.

White blood corpuscles are however found not only in the blood stream; they are also found in lymphatic vessels, where they are called lymph cells; some of the lymph cells are no doubt emigrated white blood corpuscles, but most of them are derived from lymphatic glands. The lymphatic glands are collections of lymph cells contained in a meshwork of a variety of connective tissue, called retiform tissue. It is here in fact that the lymph cells are formed from the subdivision of previously existing cells. When fully formed they work their way by means of their amœboid movements into the path of the lymph stream as it goes through the lymphatic glands. Lymphoid or adenoid tissue, i.e. tissue similar to that found in lymphatic glands, exists in many other parts, e.g. the thymus and tonsils, the solitary glands and agminated glands of the intestine, and the Malpighian corpuscles of the spleen; it forms the greater part of the corium of some mucous membranes; and it is found in microscopic patches in the lungs, liver, and other organs.

Microchemical research is obviously the only method of chemical research open when one wishes to investigate the white corpuscles in the blood. For macrochemical methods one has to obtain a supply of lymph cells from structures like lymphatic glands or the thymus; or one may use pus. In pus, however, the corpuscles cannot be regarded as normal, and have undergone certain retrogressive changes. It seems, however, quite legitimate to suppose that the white corpuscles, which are in origin lymph cells, resemble them in their chemical properties.[1] It will be convenient to take the nucleus of these corpuscles first, and then to consider the cell body or protoplasm.

The nucleus.—Like the nuclei of cells generally, the nucleus of the white corpuscle can be demonstrated to consist of a network which stains readily, and which is called chromatin, and an achromatic substance (i.e. a substance that is not easily stained by staining reagents) which has also been called nucleo-hyaloplasm (Strasburger) and para-linin (Schwarz). These and similar terms, however, which have been already more fully explained (p. 197), have more of a morphological than a chemical significance, and do not pretend to denote the chemical characters of the substances.

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[1] Wooldridge in his Arris and Gale lectures (Blood-plasma as Protoplasm, Roy. Coll. of Surgeons, 1886) pointed out certain differences between lymph cells and white blood corpuscles, in their influence upon coagulation. Such differences do not, however, affect the present argument.

The complicated manner in which the component parts of the nucleus behave to microchemical reagents must mean that the nucleus is of a complicated chemical nature, and doubtless contains many important and distinct substances.

The chief chemical substance in the nucleus of which we have other than a microscopical knowledge is nuclein. Miescher's[1] nuclein is considered by Zacharias[2] to be identical with Flemming's chromatin.

Nuclein belongs to the heterogeneous group of substances called albuminoids, i.e. substances which are not proteids, but which resemble proteids in many points. Its physical characters are like those of mucin; in containing a high percentage of phosphorus, it however differs from mucin very markedly. Its insolubility in artificial gastric juice enables us to obtain it free from the investing protoplasm of the cells. Nuclein has been obtained from many varieties of cells, from spermatozoa, from yolk of egg, milk, and also from certain plant tissues. From the discrepancies in the published analyses of these substances (by Hoppe-Seyler, Miescher, Worm-Müller, Lubavin), it seems either that no definite chemical unit nuclein exists, or that the nucleins are a numerous class of organic phosphorus compounds; this latter conclusion seems to harmonise better with the results of microchemical investigation. The investigations of Kossel,[3] in which he has shown distinct chemical differences in various kinds of nuclein, bear out this same view of the case (*see* p. 203).

The cell protoplasm.—Here again microscopic methods teach us that protoplasm is not always the uniform jelly we were once led to suppose, but consists in many cases of a fine network or *reticulum*, enclosing in its meshes a more fluid material or enchylema (Carnoy). In the white blood corpuscles the granules seem to be entangled with the reticulum.

On the application of dilute acetic acid, the granules and reticulum shrink around the nucleus. On the application of water, or more quickly with dilute potash, the protoplasm swells, and ultimately the corpuscle bursts and disintegrates. The partial disappearance of the granules when the blood is shed was observed by Haycraft to accompany the shedding out of the fibrin-ferment, or rather the formation of fibrin in the surrounding plasma.

By the use of osmic acid, fat granules, which are stained black by this reagent, can be demonstrated to exist in the cell-protoplasm, b in especial abundance in the white corpuscles and lymph cells o the intestinal vessels during absorption. The same is true with regar to glycogen, which can be detected microchemically by a solution of iodine in potassium iodide; this stains glycogen a deep mahogany colour.

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[1] Miescher, *Hoppe-Seyler's Med. Chem. Untersuch.* Heft iv. 441.

[2] *Botan. Zeitung*, 1887.

[3] Kossel, *Zeitsch. f. physiol. Chem.* x. 248.

Lecithin, cholesterin, and inorganic matter exist in small quantities in the white corpuscles, but the bulk of the protoplasm is undoubtedly proteid in nature; and though our present methods do not enable us to say which proteids are contained in the reticulum, and which in the enchylema, yet by using extracts of lymph cells from lymphatic glands we can at least identify the proteids which are present.[1] They are as follows :—

1. A mucin-like proteid similar to that described by Miescher[2] in pus, and called hyaline substance by Rovida. This swells up into a jelly-like substance when mixed with 5 to 10 per cent. solutions of sodium chloride or magnesium sulphate; on pouring such a mixture into water, this proteid extends in cohesive strings through the water, which soon contract and float on the top. This substance is, however, not mucin, as it yields no reducing sugar on boiling it with sulphuric acid. It is also not nuclein, as the nuclei are not affected by the reagents used; it resembles globulins in its solubilities; it yields an ash rich in phosphorus; and on digestion with artificial gastric juice an insoluble residue of the nature of nuclein separates out. In all these points this proteid resembles the class of proteids named '*nucleo-albumins*' by Hammarsten.[3] This is the most abundant of the proteids present in the protoplasm. It is probably identical with Reinke's plastin (*see* p. 205).

2. Two globulins. These are obtained by dissolving the proteids of the lymph cells in a liquid prepared by mixing a saturated solution of sodium sulphate with nine times its volume of distilled water. This solution does not cause the swelling up of the nucleo-albumin like sodium chloride or magnesium sulphate solutions do. Then on saturating this extract with magnesium sulphate a precipitate is obtained. This precipitate consists of the globulins, which may be washed, redissolved, and then separated by fractional heat-coagulation. The may be called *cell-globulin α*, which coagulates at about 50° C.; an *cell-globulin*, which coagulates at 73° C. On filtering off the heat coagulum of cell-globulin *α*, which is generally only present in sma quantities, the cell-globulin proper is alone left in solution, and th

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[1] *Reports of the British Association*, 1887, p. 145, and 1888, p. 363. Report of Committee appointed to investigate the Physiology of the Lymphatic System.

[2] Miescher, *Hoppe-Seyler's Med. Chem. Untersuch.* p. 441.

[3] Hammarsten, *Zeitsch. f. physiol. Chem.* xii. 163.

has the properties of fibrin-ferment. Reasons have already been given for considering the fibrin-ferment and cell-globulin as identical (*see* p. 241).

3. An albumin. After filtering off the globulins, the albumin remains in solution. It coagulates at 73° C. and resembles serum-albumin *a* in its properties. It is present in very small quantities, and may be provisionally termed *cell-albumin*.

In concluding this account of the proteids of lymph cells, it may be added that no substance like myosin or fibrin can be obtained from the cells; there is, however, a formation of sarkolactic acid[1] after death as in muscle : and if the glands be left, especially at the temperature of the body, for some hours after death, a process of self-digestion takes place, the pepsin present in the glands, as it is in most tissues (Brücke), becoming active when the reaction of the tissue becomes acid ; under these circumstances there is, in addition to the proteids already enumerated, a small and varying amount of proteoses and peptones.

The nucleo-albumin was mistaken by some of the earlier observers for myosin, from which it differs markedly. Some also have mistaken it for fibrin[2] ; the way in which it extends in strings when poured into water accounts for this ; these strings subsequently contract, and here indeed is a point of resemblance between it and fibrin. But here all resemblance stops.

THE BLOOD TABLETS

In addition to the white and red corpuscles, a number of colourless discs averaging ·002—·003 millimetre diameter are also seen. They exist as such in the circulating blood. By some they have been supposed to be stages in the development of red corpuscles ; some[3] consider them to be masses of undifferentiated protoplasm, but their origin and destiny has never been explained. The action of inert solids upon them after the blood is shed is much the same as on white blood corpuscles. It causes them to become sticky, to run together, lose contour, change shape, and in many cases undergo complete disintegration. Strands of fibrin start from collections of blood-plates, so probably one product at least of their disintegration is fibrin-ferment.

In spite of the large amount of research from the histological standpoint, on these blood tablets (Blutplättchen of Bizzozero[1]), we know virtually nothing of them chemically.

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[1] It was Hirschler who showed that the variety of lactic acid formed was sarkolactic acid (*Zeitsch. f. physiol. Chem.* xi. 41). Berlinerblau (*Chem. Centralbl.* 1888, p. 757) states that lactic acid is a normal constituent of blood. But Salomon (*Virchow's Archiv*, cxiii. 356) has shown that fresh blood contains no lactic acid, but on standing a small amount forms, no doubt from changes of a fermentative nature in the white corpuscles.

[2] Denis called it fibrine concrète globuline. Wooldridge also spoke of it as fibrin (*Du Bois Reymond's Arch. f. Physiologie*, 1881, pp. 387–411). Hammarsten was the first to show that it is not true fibrin (*see* p. 282).

[3] Haycraft, *Journ. of Anat. and Physiol.* xxii. 302.

The term hæmatoblasts has been applied by some to the blood tablets; this is liable to cause confusion, as the same word is used for the nucleated red corpuscles which occur in certain stages of the formation of the non-nucleated red discs of vertebrates.

The blood tablets are found only in mammals' blood; in fishes, birds, and amphibians they are absent, and according to Eberth and Schimmelbusch,[2] and also Hayem,[3] their place is taken in these groups by certain spindle-shaped nucleated cells. Löwit[4] on the other hand regards the spindle cell as a variety of white blood corpuscle, and the blood tablets of mammals as something peculiar to that group; according to him they consist chiefly of a globulin, and he considers they play an important part in the formation of fibrin.

THE RED BLOOD CORPUSCLES

The red or coloured corpuscles give the red appearance to the blood. They are much more numerous than the white corpuscles, there being about 5,000,000 per cubic millimetre in the human male, about 4,500,000 in the female.

The enumeration of the blood corpuscles is readily effected by the hæmacytometer of Gowers.[5] This instrument consists of a glass slide (fig. 54 C), the centre of which is ruled into $\frac{1}{10}$ millimetre squares and surrounded by a glass rim $\frac{1}{5}$ millimetre thick. It is provided with measuring pipettes (A and B), a vessel (D) for mixing the blood with a saline solution (sulphate of soda of specific gravity 1015),[6] a glass stirrer (E), and a guarded needle (F).

The mode of proceeding is extremely simple. 995 cubic millimetres of the saline solution are measured out by means of A, and then placed in the mixing jar; 5 cubic millimetres of blood are then drawn from a puncture in the finger by means of the pipette B, and blown into the solution. The two fluids are well mixed by the stirrer, and a small drop of this diluted mixture placed in the centre of the slide C; a cover glass is gently laid on (so as to touch the drop, which thus forms a layer $\frac{1}{5}$ mm. thick between the slide and cover glass), and pressed down by two brass springs. In a few minutes the corpuscles have sunk to the bottom of the layer of fluid, and rest on the squares. The number on ten squares is then counted, and this multiplied by 10,000 gives the number in a cubic millimetre of blood. The average number of red corpuscles in each square ought, therefore, in normal human blood to be 45–50.

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[1] Bizzozero, *Virchow's Archiv*, xc. 261.

[2] *Virchow's Archiv*, cviii. 366.

[3] Hayem, *Du sang*, Paris, 1889.

[4] *Archiv f. exp. Path. u. Pharmakol.* xxiv. 188.

[5] Gowers, *Lancet*, Dec. 1, 1877. Malassez (*Compt. rend.* 1872) and Hayem (*Du sang*) have invented very similar instruments.

[6] There are many similar saline solutions which may be employed.

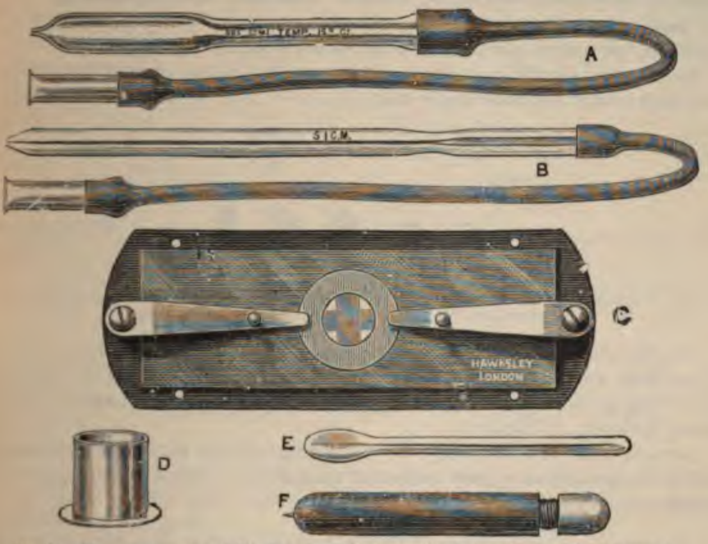

FIG. 54.—Hæmacytometer of Dr. Gowers. (Made by Hawksley & Co., Oxford Street.)

Specific gravity.—C. Schmidt gives the specific gravity of red blood corpuscles as 1·089, Welcker as 1·105.

Shape and size.—They vary in size and structure in different groups of the vertebrate sub-kingdom. In Mammalia, with the exception of the Camelidæ, they are biconcave, circular discs ; they have no nucleus except during embryonic life, and they have a tendency to run into rouleaux when the blood is at rest, but if it is disturbed they readily become separated. In the Camel tribe they have an elliptical outline. Their average diameter in mammals is ·007–·008 millimetre[1] ($\frac{1}{3200}$ inch); and about one-fourth of that in thickness ; there are very slight variations in different classes of mammals. In birds, reptiles, amphibians, and fishes, the red corpuscles are biconvex, oval discs, with a nucleus ; they are largest in the amphibia.

Action of microscopic reagents.—*Water* causes the corpuscles to swell up, and at the same time dissolves out the hæmoglobin, leaving a globular colourless stroma.

Salt solution causes the corpuscles to shrink. They become wrinkled or crenated on the surface. The action of water and of salt solution suggests the existence of a membrane on the surface of the corpuscle, through which osmosis takes place. The question, has the red corpuscle a membrane? was once the subject of voluminous discussion. An admirable summary of the positions held by the older writers is given in Gamgee's 'Physiological Chemistry' (p. 72). The matter has been finally compromised by considering the stroma to be rather denser at the surface than in the interior. The outer denser part plays the rôle of a membrane during osmotic phenomena.

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[1] This is often written 7–8μ. 1μ (micro-millimetre) = one-thousandth of a millimetre.

Dilute alkalis (0·2 per cent. potash) slowly dissolve the corpuscles.

Dilute acids (1 per cent. acetic acid) act like water; and in nucleated red corpuscles render the nucleus distinct.

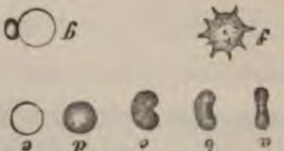

FIG. 55.—*a–e*, successive effects of water on a red blood corpuscle; *f*, a red corpuscle crenated by salt solution; *g*, action of tannin on a red corpuscle.

Tannic acid causes a discharge of hæmoglobin from the stroma, but this is immediately altered and precipitated. It remains for a short time adherent to the stroma in the form of a round or irregular globule of a brownish tinge, consisting probably of hæmatin.

Boracic acid acts similarly, but in nucleated red corpuscles the colouring matter is partially or wholly collected around the nucleus, which may then be extruded from the corpuscle.

Nucleus.—The nucleus of those red corpuscles in which one exists, has the usual reticular structure, and consists according to Lauder Brunton[1] and Plósz[2] of nuclein. Defibrinated blood from the bird was treated with ten or twelve times its volume of 3 per cent. sodium chloride solution, and the corpuscles separated by decantation. On shaking the corpuscles with a mixture of water and ether, the nuclei alone remain undissolved and float at the junction of the two liquids.

Nuclein may, however, also be prepared from red corpuscles by Miescher's method, which consists in subjecting the corpuscles to artificial gastric digestion. The nuclei alone remain undigested.

Origin of blood corpuscles in mammals.—The following is a brief résumé of the chief ascertained facts concerning the origin of the red discs[3]:—

In the embryo the first formed coloured blood corpuscles are amœboid nucleated cells. These are developed within certain mesoblastic cells which are united to form a network. The nuclei of the cells multiply, and around some of them, protoplasm coloured by hæmoglobin is aggregated. Finally the network is hollowed out and filled with fluid; thus capillaries are produced; the coloured nucleated portions of protoplasm are set free within these as the embryonic blood corpuscles. In later embryonic life these are replaced by the usual non-nucleated discs, which are moulded within connective tissue cells as before, except that the cell nuclei do not participate in the process.

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[1] L. Brunton, *Journ. of Anat. and Physiol.* 2nd series, vol. iii. p. 91.

[2] Plósz, *Hoppe-Seyler's Med. Chem. Unters.* Heft iv. p. 460.

[3] *Quain's Anat.* vol. ii.

Nucleated coloured corpuscles are not seen in the blood after birth; but they continue to be formed in the red marrow, and in some animals in the spleen also. Probably the nucleus disappears from them, and the coloured protoplasm is moulded into a discoid shape. Malassez, however, considers that the red discs are formed by a process of budding from these cells, which he terms globuligenic cells.[1]

The evidence that the red corpuscles are derived from the white, or from the nuclei of the white corpuscles, or from the blood tablets, is insufficient.

Composition.—According to C. Schmidt, 1000 parts of moist red corpuscles contain:—

Water		688 parts.
Solids	Organic	303·88 „
	Mineral	8·12 „

According to Hoppe-Seyler and Jüdell,[2] 100 parts of dried corpuscles contain:—

	Human Blood		Dog's Blood	Goose's Blood
	I.	II.		
Proteids	12·24	5·10	12·55	36·41
Hæmoglobin	86·79	94·30	86·50	62·65
Lecithin	0·72	0·35	0·59	0·46
Cholesterin	0·25	0·25	0·36	0·48

The mineral constituents of the red corpuscles have been investigated by C. Schmidt, and the following tables contrast those of the red corpuscles with those of the plasma in man.

1000 parts of moist corpuscles yield:—

Mineral matter (exclusive of iron, which is contained in the hæmoglobin)	8·120
Chlorine	1·686
Sulphuric anhydride	0·066
Phosphorus pentoxide	1·134
Potassium	3·328
Sodium	1·052
Calcium phosphate	0·114
Magnesium phosphate	0·073

1000 parts of plasma yield:—

Mineral matter	8·550
Chlorine	3·640
Sulphuric anhydride	0·115
Phosphorus pentoxide	0·191
Potassium	0·323
Sodium	3·341
Calcium phosphate	0·311
Magnesium phosphate	0·222

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[1] McKendrick's *Physiology*, vol. ii. p. 170.

[2] Hoppe-Seyler and Jüdell, *Med. Chem. Untersuch.* Heft iii. p. 386. P. Manasse ...t. *physiol. Chem.* xiv. 452), gives the percentage of lecithin in the red corpuscles as 7, of cholesterin as 0·151.

The remarkable difference in the distribution of potassium and sodium seen in the above does not, however, hold for most animals, as the following table shows (Gamgee)[1] :--

	Blood Cells			Liquor Sanguinis		
	K	Na	Cl	K	Na	Cl
Man	40·89	9·71	21·00	5·19	37·74	40·68
Dog	6·07	36·17	24·88	3·25	39·68	37·31
Cat	7·85	35·02	27·59	5·17	37·64	41·70
Sheep	14·57	38·07	27·21	6·56	38·56	40·89
Goat	37·41	14·98	31·73	3·55	37·89	40·41

Oxygen is contained in combination with the hæmoglobin to form oxyhæmoglobin. The corpuscles also contain a certain amount of carbonic acid (*see* Respiration).

The chief constituent of the corpuscles is thus hæmoglobin.

According to Hoppe-Seyler[2] lecithin exists as such in the red corpuscles ; earlier observers (Liebreich, Hermann[3]) considered that it is present in the form of a substance called protagon ; of which lecithin is a decomposition product. Protagon is according to Hoppe-Seyler a mere mixture of lecithin and cerebrin. Gamgee,[4] however, has more recently shown that protagon is a perfectly definite proximate principle, and probably exists in nervous tissue as such, though with regard to the red corpuscles Hoppe-Seyler's view is now generally held. Lecithin and cholesterin are extracted from the corpuscles by ether. They will be more fully described under nervous tissue.

The proteids of the stroma.—The best method for preparing the stromata of the corpuscles is that of Wooldridge[5] ; defibrinated blood is centrifugalised repeatedly with a 1 per cent. solution of sodium chloride until the corpuscles are obtained free from adherent serum ; they are then dissolved in 5 or 6 times their volume of water, and shaken with a little ether to assist the solution ; the white corpuscles are then allowed to settle, or may be separated by centrifugalising. From this solution the stromata are precipitated by the addition of a few drops of a 1 per cent. solution of acid sodium sulphate. The precipitate is collected, washed, and may be readily dissolved in a 5 per cent. solution of magnesium sulphate. Kühne,[6] who used a rather different method of separating the stromata, found that their chief proteid constituent was a fibrino-plastic globulin. This result I have

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[1] Gamgee, *Physiol. Chem.* p. 122.

[2] Hoppe-Seyler, *Med. Chem. Untersuchungen*, Heft i. p. 140. Jüdell, *Ibid.* iii. 386.

[3] Hermann, *Archiv f. Anat. u. Physiol.* 1866, p. 83.

[4] Gamgee and Blankenhorn, *Journ. of Physiol.* 1879. Gamgee, *Physiol. Chem.* p. 83.

[5] *Du Bois Reymond's Archiv f. Physiol.* 1881, p. 387.

[6] *Lehrbuch*, p. 193.

(working with Dr. Friend[1]) been able to fully confirm. The chief proteid present is cell-globulin, and like that obtained from white corpuscles it is apparently identical with fibrin-ferment; though whether the cell-globulin of the red corpuscles normally takes any active part in producing coagulation appears to me to be very doubtful; it may perhaps do so under certain circumstances, accounting for what Landois terms 'stroma-fibrin.' The stromata contain also a doubtful trace of cell-albumin; but the nucleo-albumin described in white corpuscles (p. 260) is entirely absent from the red.

Hæmoglobin

Hæmoglobin is the red pigment of the coloured corpuscles. It is a substance which gives the reactions of a proteid, but differs from other proteids in containing the element iron, and in being crystallisable.

It exists in the blood in two conditions: in arterial blood it is combined loosely with oxygen, and is called oxyhæmoglobin; the other condition is the deoxygenated or reduced hæmoglobin (often called simply hæmoglobin) which occurs in venous blood, that is, the blood which is returning to the heart after it has supplied the tissues with oxygen. Hæmoglobin is thus the oxygen carrier of the body, and it may be called a respiratory pigment.

Distribution.—Hæmoglobin is by far the most widely distributed of the respiratory pigments. It occurs in special corpuscles in all vertebrates except *Amphioxus* and *Leptocephalus* (Lankester)[2]; in the following crustaceans—*Daphnia*, *Cheirocephalus* (Lankester),[3] *Apus*, *Cypris* (Regnard and Blancard),[4] *Lernanthropus*, *Clavella*, and a marine parasitic crustacean (undescribed) (Van Beneden)[5]; in the following insects—*Cheironomus* (Lankester),[3] *Musca domestica* (MacMunn)[6]; in the following molluscs—*Planorbis*, *Arca*, and *Solen* (Lankester)[3]; in the following chætopod worms—*Lumbricus*, *Lumbriculus*, *Limnodrilus*, *Eunice*, *Cirrhatulus*, *Nais*, *Nereis*, *Terebella*, *Glycera*, *Chætogaster*, *Capitella*, *Tubifex*, *Arenicola*, *Enchytrachus*, and *Aphrodite* (Lankester)[3]; in the following gephyrean worms—*Phoronis*, *Thallasema*, and *Hamingia* (Lankester)[3]; in the nemertine worm *Polia* (Lankester), and others of the same class (Hubrecht)[5]; in the leeches *Nephelis* and *Hirudo* (Lankester); in an ophiurid echinoderm (Fottinger); and in a holothurian (Howell).[7] In the above cases, however, from the invertebrate kingdom, the hæmoglobin does not occur in special corpuscles, but is simply in solution in the blood plasma, which has thus a respiratory, in addition to its nutritive functions. There are, however, eight invertebrate animals in which this is not the case, but coloured corpuscles exist as in vertebrates; these are the two molluscs, Solen and Arca, and the five worms, Glycera, Capitella, Phoronis, Thallasema, and Hamingia, and the holothurian Thyonella.

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[1] *Journ. of Physiol.* x. 532.

[2] Lankester, *Proc. Roy. Soc.* xxi. 1872, p. 71.

[3] Lankester, *Journ. of Anat. and Physiol.* ii. 114; *Pflüger's Archiv*, iv. 315.

[4] Regnard and Blancard, *Zool. Anzeig.* 1883, p. 253.

[5] Quoted by Lankester, *Zool. Anzeig.* 1883, p. 416, and by Gamgee, *Physiol. Chem.* p. 130.

[6] MacMunn, 'Animal Chromatology,' *Proc. Birmingham Philosophical Society*, vol. iii. p. 385.

[7] *Studies from the Johns Hopkins Univ. Baltimore* (*Biol. Lab.*), vol. iii. p. 284.

Hæmoglobin occurs not only in the blood, but it is present in certain muscles, especially the red muscles of rodents; and it also occurs in the muscles of certain invertebrates, even in some of those in which it is not present in the blood (Lankester). (*See* Muscle.)

Hæmoglobin occurs also in the nerve cells of Aphrodite. (*See* Nerve.)

Hæmoglobin may occur in the urine and other fluids where it is normally absent. (*See* Urine—Hæmoglobinuria.)

Preparation of oxyhæmoglobin crystals from blood.—The following will be found the best methods for the preparation of oxyhæmoglobin crystals.[1]

1. Defibrinated blood is mixed with its own volume of distilled water, and the diluted fluid is heated with one-fourth of its volume of alcohol. The mixture is kept for 24 hours at a temperature of 0° C. or below. The crystals which separate are dissolved in a little water, a fourth of its volume of alcohol added, and the mixture again frozen. To obtain a pure product the process of recrystallisation may be several times repeated.

2. Defibrinated blood is shaken with one-sixteenth of its volume of ether; the corpuscles dissolve, and the blood assumes a *laky* appearance. After a period varying from two minutes to three days, a thick magma of crystals has formed; these are washed by decantation and centrifugalisation with 25 per cent. alcohol. They may then be redissolved and recrystallised as in 1. This method is by far the most satisfactory one.

3. Zinoffsky[2] uses ammonia instead of ether to dissolve the stromata in the foregoing method; this has subsequently to be neutralised with hydrochloric acid.

4. Gschleidlen[3] obtains large crystals from dog's blood by sealing the defibrinated liquid, after it has stood in the air for 24 hours, in capillary tubes. These are kept at 37° C. for some days, and then their contents are poured out into a watch-glass. Crystals then form on evaporation.

5. In the blood of some animals (rat, guinea-pig, squirrel), microscopic preparations of the crystals may be obtained by simply mixing a drop of the defibrinated blood with a drop of water on a slide; a cover-glass is then put on, and in a few minutes the corpuscles are

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[1] The first two methods are selected from a number of methods described by Gamgee in his *Physiological Chemistry*, pp. 85–89.

[2] Zinoffsky, *Zeit. physiol. Chem.* x. 16.

[3] Gschleidlen, *Physiolog. Methodik*, p. 361.

rendered colourless, and then the oxyhæmoglobin crystallises out from the solution so formed.[1]

6. More permanent preparations may be made by Stein's [2] method, which consists simply in mounting a drop of blood in a drop of Canada balsam on a slide and covering it. In a few minutes crystals form.

Crystals of reduced hæmoglobin have been prepared by similar methods to those already described; but oxygen must be carefully excluded during the experiments (Hüfner,[3] Nencki and Sieber [4]).

Blood crystals are obtained with greater difficulty from the blood of some animals than from that of others. Preyer thus classifies these varieties of blood according to facility of crystallisation:—

1. Very difficult: calf, pig, pigeon, frog.
2. Difficult: man, ape, rabbit, sheep.
3. Easy: cat, dog, mouse, horse.
4. Very easy: rat, guinea-pig.

From my own experiments I should be inclined to put the mouse in the second class, and add the squirrel to the fourth class in the above list.

The crystals differ also in solubility in water and other reagents, which is in the inverse ratio to their facility of crystallisation.

The oxyhæmoglobin crystals from different animals differ slightly in their percentage composition.

The oxyhæmoglobin differs also in the amount of water of crystallisation with which it combines.

Oxyhæmoglobin is by the action of acids and alkalis decomposed, and a brown pigment called hæmatin formed. The readiness with which this decomposition is brought about, also differs in different animals; e.g. in the blood of the dog and man it occurs easily, in that of herbivorous animals with difficulty (Körber,[5] Krüger [6]).

Lastly the blood crystals differ in form. As a rule they are rhombic

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[1] On watching this process some of the corpuscles appear to set into minute hexagons or other shaped crystals; this apparently is what Preyer called intraglobular crystallisation. This is, however, not true crystallisation, but simply a partial crenation of the corpuscle; if any of the blood has been allowed to dry it may be well seen. Under the subsequent action of water, the corpuscle swells, and its resemblance to a crystal disappears.

[2] Stein, *Centralbl. f. d. med. Wiss.* 1888, No. 23, and *Virchow's Archiv*, xcvii. 483.

[3] Hüfner, *Zeit. physiol. Chem.* iv. 382.

[4] Nencki and Sieber, *Berichte der deutsch. chem. Gesell.* xix. 128 *Ibid.* p. 410. Copeman (*Brit. Med. Journ.* ii. 1889, p. 190) states that the crystals which he has obtained from human blood by adding putrid serum to it are always composed of reduced hæmoglobin.

[5] Körber, *Inaug. Dissert.* Dorpat, 1866.

[6] Krüger, *Zeit. Biol.* xxiv. 318.

prisms ; in the squirrel and hamster [1] hexagons [2] ; and in the guinea-
and certain birds rhombic tetrahedra.

Oxyhæmoglobin crystals thus differ in the following points :

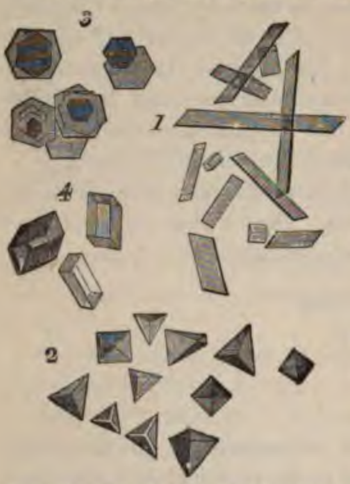

FIG. 56.—Oxyhæmoglobin crystals magnified : 1, from human blood ; 2, from the guinea-pig ; 3, squirrel ; 4, hamster.

1. Readiness of crystallisation.
2. Solubility.
3. In percentage composit
(slightly).
4. Amount of water of crysta
sation.
5. Readiness to undergo dec
position by acids or alkalis.
6. Crystalline form.

In spite of this, however,
hæmoglobin is universally the s
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1. Spectroscopic properties.
2. The compounds it forms.
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We shall approach the question best by dealing at greater len
with the

Crystallography of Oxyhæmoglobin

Oxyhæmoglobin crystals were first described by Reichert[3] as occurring in
uterus of a pregnant guinea-pig ; by Leydig[4] as occurring in the alimentary c
of the leech ; and by Kölliker,[5] obtained from the blood of the dog, python,
other animals. Kölliker considered the crystals to be composed of a more or
modified hæmatin. Funke[6] was, however, the first to make complete observati

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[1] In the hamster rhombohedra are also found.

[2] Bojanowski (*Zeitsch. f. wiss. Zool.* xii. 1863, 333) says that the blood crys
of the mouse are also hexagonal. This I have not been able to confirm ; but have fo
with Kunde that they are rhombic. Still it is possible that they may be someti
hexagonal. Rat's hæmoglobin is also sometimes hexagonal when prepared by Ste
method. This was first pointed out to me by Dr. Sheridan Lea (*see* more fully *Qu
Journ. of Mic. Science*, xxviii. 190). Hüfner and Bücheler obtained in one case hexag
oxyhæmoglobin crystals from horse's blood (*Zeit. physiol. Chem.* viii. 358).

[3] Reichert, *Müller's Archiv*, 1849, p. 197.

[4] Leydig, *Zeitsch. f. wiss. Zool.* Bd. i. 1849, p. 116.

[5] Kölliker, *Zeitsch. f. wiss. Zool.* Bd. i. 1849, p. 266.

[6] Funke, *Zeitsch. f. rat. Med.* N. F. Bd. i, 1851, p. 184 ; Bd. ii. 1852, p. 204
p. 288. *De sanguine venæ lienalis, Diss. Lipsiæ*, 1851.

upon them, and to recognise their true nature. Kunde,[1] working at the same time, made extensive observations from a comparative point of view, and was the discoverer of the exceptional form of the crystals in the guinea-pig and squirrel. Since then, many investigators have worked at the subject, notably Lehmann,[2] Rollett,[3] von Lang,[4] and Preyer,[5] who has written an exhaustive treatise on the subject.

The tetrahedral blood crystals of the guinea-pig were at one time supposed to belong to the regular system, but it was von Lang who showed that they are in reality rhombic.

A similar question might arise with regard to the hexagonal crystals of the squirrel and the hamster. May they not be rhombic crystals which have what mineralogists call a hexagonal habit[6] (*see* fig. 57 *a*)? or might they not be rhombic twins consisting of three parallelograms or six triangles (as shown in fig. 57 *b* and *c*)?

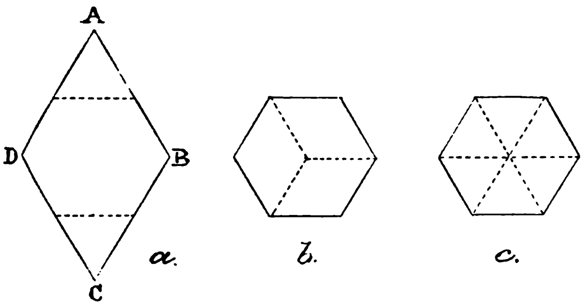

Fig. 57. Suppose A B C D (*a*) to be the basal plane of a rhombic plate, and the angle A B C to be approximately 120°, the lines joining A C, B D being the axes. Then if the angles D A B, D C B be replaced, as shown by the dotted lines, a hexagon will be produced differing but little from a regular hexagon.

In order to settle this question it is necessary to examine the optical properties of the crystals.

Crystals may be divided, according to their optical properties, into three classes:—

1. *Isotropic.*—Those in which there is no distinction of different directions as regards optical properties. This includes crystals belonging to the regular system. They have but one refractive index, i.e. refract light, like amorphous bodies, singly.

2. *Uniaxal.*—Those in which the optical properties are the same for all directions equally inclined to one particular direction, called the optic axis, but vary according to this inclination. This class includes crystals belonging to the dimetric system (crystals with three rectangular axes, two of them being equal) and the hexagonal system. The optic axis corresponds with the principal crystallographic axis; that is, in the case of a hexagon the axis perpendicular to

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[1] Kunde, *Zeitsch. f. rat. Med.* N. F. Bd. ii. 1852, p. 276.

[2] Lehmann, *Ber. d. k. sächs. Ges. d. Wissen.* 1852, p. 22.

[3] Rollett, *Sitzungsber. d. Wien. Akad.* Bd. xlvi. 1862, p. 65.

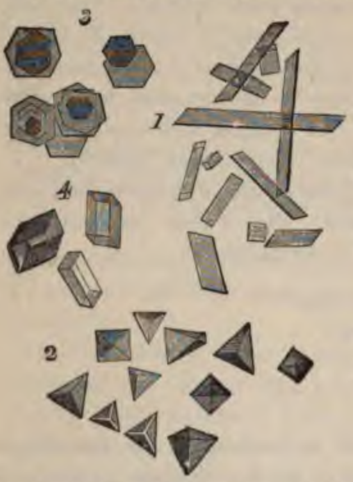
[4] Lang, *Ibid.*

[5] Preyer, *Die Blutkrystalle*, Jena, 1871.

[6] Copper glance is an instance of this occurring in the mineral kingdom. In one form of mica also, crystals of the monoclinic system simulating hexagons are found.

prisms ; in the squirrel and hamster[1] hexagons[2] ; and in the guinea-p and certain birds rhombic tetrahedra.

Oxyhæmoglobin crystals thus differ in the following points :



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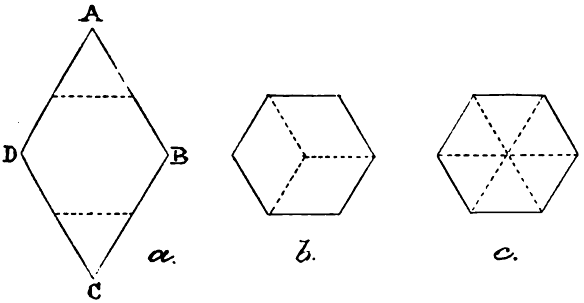

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Oxyhæmoglobin crystals thus differ in the following points :

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2. Solubility.
3. In percentage composit (slightly).
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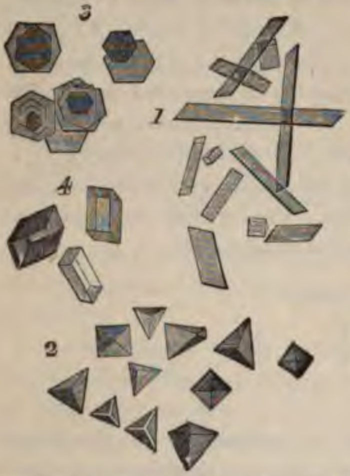

FIG. 56.—Oxyhæmoglobin crystals magnified ; 1, from human blood ; 2, from the guinea-pig ; 3, squirrel ; 4, hamster.

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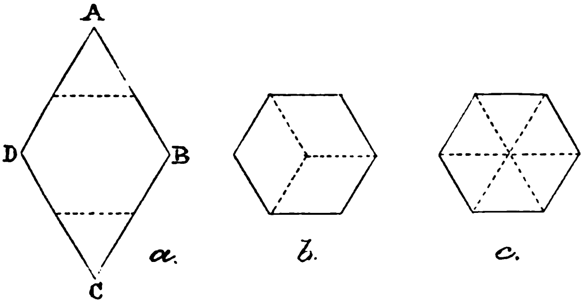

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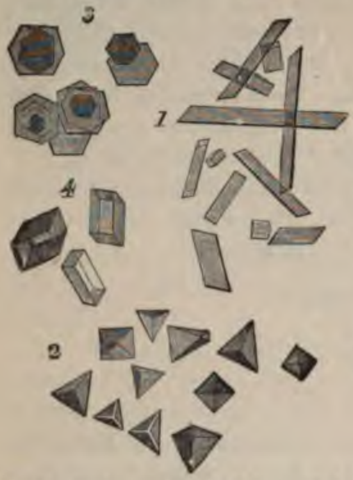

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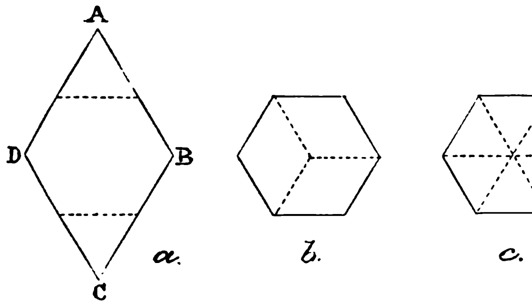

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the flat surface. In the direction of this axis a ray of light is refracted singly, and in other directions doubly.

3. *Biaxal.*—This includes the remaining three systems of crystals, the trimetric or rhombic (three rectangular axes all unequal), the monoclinic, and the triclinic. In these there are always two directions along which a ray is singly refracted.

The best test as to whether a substance is doubly refractive or not is this: If between crossed nicols, which consequently appear dark, a substance be interposed that makes the darkness give place to illumination, however feeble, that substance is doubly refractive. This action is termed the depolarisation of the ray (*see* p. 38).

On submitting the squirrel's blood crystals to this test, they are found to remain dark in the dark field of the polarising microscope when they are examined with the apparent basal plane perpendicular to the axis of the instrument and rotated; nor when a quartz plate is inserted, do they produce any modification of the tint as the stage is turned.

Hence the presumption is, that they belong to the hexagonal system, as rhombic crystals of hexagonal habit or rhombic twins would produce some double refraction when examined in this way.

It is generally stated that blood crystals are doubly refracting and pleochromatic (i.e. exhibit tints as the upper nicol is rotated). We see it is necessary to make an exception to this rule in the case of hexagonal plates when lying flat.

It is found that the hexagonal crystals from squirrel's blood are too small and thin to allow of one applying the additional crucial test of the interference figures seen in convergent polarised light. These consist of a cross and circles, which are symmetrical in uniaxal crystals, asymmetrical in biaxal crystals.

We have, however, in the case of the hamster the occurrence of rhombohedral crystals; this confirms the view that the crystals are true hexagons, as the rhombohedron belongs to the hexagonal system.

It is found, however, that after recrystallising[1] squirrel's oxyhæmoglobin several times, their hexagonal constitution is broken down, and the crystals obtained are either rhombic prisms or a mixture of these with rhombic tetrahedra.[2] This leads us to believe that whatever the difference between the various forms of oxyhæmoglobin may be, it cannot be a very deep or essential one.

Have we then to deal with a case of polymorphism? The terms dimorphism and polymorphism cannot be applied to any substance which crystallises in two or more forms, unless the composition of that substance be exactly the same in all cases. Instances of dimorphism in the mineral world are carbon and sulphur among the elements, and sal ammoniac, potassium iodide, &c., among compounds. The conditions on which dimorphism depends are two: first, temperature; secondly, the solvent from which the substance crystallises. If, as in the case of many mineral salts, the compounds are united with different proportions of water of crystallisation, we have to deal with different hydrates, and the case is not one of true dimorphism; an instance of this is sulphate of soda.

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[1] Another peculiar result of recrystallising hæmoglobin has been pointed out by Kupffer and more recently by Krüger (*Zeit. Biol.* xxiv. 47), that is, that the absorption coefficient of oxyhæmoglobin increases after recrystallisation. In determining the absolute amount of oxyhæmoglobin by the spectrophotometer (*see* p. 50) it is best only to recrystallise once, as each recrystallisation increases the error of observation.

[2] Halliburton, *Quart. Journ. Mic. Science*, xxviii. 181. Some remarkable forms of oxyhæmoglobin crystals are also sometimes obtained by dissolving a mixture of the hæmoglobin of various animals and then crystallising.

The case seems to me to narrow itself down to this in the case of hæmoglobin; either we have here a case of polymorphism, or the crystalline forms are due to the combination with varying proportions of water of crystallisation. In the absence of a rational formula for hæmoglobin, it would be unsafe to affirm the former of these two alternatives. Moreover, the conditions that are known to produce dimorphism in minerals, namely, differences of temperature and of solvent, have in the case of hæmoglobin no influence.

If we then fall back on the latter alternative, the question which arises is whether there are any facts to support it. The explanation that the varying form of oxyhæmoglobin is due to varying quantities of water of crystallisation may be otherwise expressed by saying that we have to deal with *different hydrates of oxyhæmoglobin.* This would account for the varying solubilities of these substances in water and other reagents, and at the same time is not such an essential difference as to prevent the chief properties of oxyhæmoglobin from being universally the same.

Turning to Hoppe-Seyler's researches on this subject of water of crystallisation, it is seen that its amount varies considerably. The following is his table:—[1]

	Percentage of Water of Crystallisation
Dog's hæmoglobin	3 to 4
Guinea-pig's ,,	7
Squirrel's ,,	9·4
Goose's ,,	9·4

In an earlier paper,[2] the same author gives rather different percentages, viz. for guinea-pig's hæmoglobin 6, for goose's hæmoglobin 7, and for squirrel's hæmoglobin 9. C. Bohr[3] has more recently made observations on the water of crystallisation of dog's hæmoglobin, and as the result of thirteen experiments he finds that its amount varies from 6·3 to 1·2 per cent. It is thus seen that great variations occur in the numbers obtained by these experiments.[4] The reason for this variation seems to be the great difficulty of obtaining hæmoglobin in a pure state, and also possibly because the method adopted, which is the same as that carried out in similar investigations on inorganic salts, is not applicable to such a complex and much less stable organic compound as hæmoglobin; in other words, the temperature necessary to drive off the water of crystallisation is also sufficient to cause certain decomposition changes in the pigment.

My experiments have shown that squirrel's oxyhæmoglobin will under certain circumstances crystallise in forms other than the usual hexagonal form. A crucial experiment in order to see whether this is due to union with different amounts of water of crystallisation would have been first to ascertain the amount of this water in the hexagonal crystals, and then in the rhombic crystals obtained by recrystallisation. I have performed three such experiments, but the results obtained are conflicting, and exhibit variations as great as in Bohr's experiments, so that it is impossible to draw any conclusions from them, except the negative one that we cannot by our present methods of research make any definite statement with regard to the water of crystallisation of oxyhæmoglobin.

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[1] *Physiologische Chemie*, p. 377.

[2] *Med. Chem. Untersuchungen*, Heft iii. 1868, p. 370.

[3] *Experimentale Untersuchungen über die Sauerstoffaufnahme des Blutfarbstoffes*, Kopenhagen (Olsen and Co.), 1885.

[4] The same difficulty in obtaining concordant results in the estimation of water of crystallisation was found by J. G. Otto, *Pflüger's Arch.* xxxi. 240.

Even if it be found ultimately that the difference in crystalline form is dependent on varying amounts of water of crystallisation, the difficulty is only explained up to a certain point. What is left unexplained is the nature of the agency that causes the oxyhæmoglobin of some animals to unite with a certain amount of water of crystallisation, and that of other animals with a different amount. That some such substance or agency does exist would seem to be the inevitable result of the recrystallisation experiments which have been related. It may, however, be stated that this part is not played by any constituent of the serum. The corpuscles of one animal may be obtained free from serum by centrifugalising and then mixing with the serum of some other animal whose blood crystals have another form. But it is found on subsequent crystallisation that the characteristic form of the blood crystals is not altered thereby. One can only suggest that it is some constituent of the stroma which exerts the influence in question.

Compounds of Hæmoglobin

Hæmoglobin forms at least four compounds with gases, viz.:

With oxygen : 1. Oxyhæmoglobin.
2. Methæmoglobin.
With carbonic oxide : 3. Carbonic oxide hæmoglobin.
With nitric oxide : 4. Nitric oxide hæmoglobin.

These compounds are isomorphous, they have similar crystalline forms; they each consist probably of a molecule of hæmoglobin combined with one of the gas in question. They part with the combined gas somewhat readily; but they are arranged in order of stability in the above list, the least stable first.

1. *Oxyhæmoglobin.*—This is the compound which exists in arterial blood. Many of its properties have been already mentioned. The oxygen linked to hæmoglobin, which is removed by the tissues through which the blood circulates, may be called *the respiratory oxygen of hæmoglobin.* The circumstances under which hæmoglobin combines with and parts from its respiratory oxygen in the body will be fully described under 'Respiration.' But the same processes may be imitated outside the body, using either blood or pure solutions of hæmoglobin. The respiratory oxygen can be removed, for example, in the Torricellian vacuum of an air pump. Preyer[1] estimated that 1 gram of hæmoglobin will combine with 1·27 c.c.[2] of oxygen. Hüfner[3] gives almost the same number, viz. 1·28 c.c. of oxygen.

A. Schmidt at one time considered that the respiratory oxygen of hæmoglobin was ozonised, and therefore more active than atmospheric oxygen. Pflüger[1] has shown that this is not the case. When diluted blood is dropped on a filter paper which has been moistened with tincture of guaiacum and then dried, a blue ring sometimes forms at the edge of the drop. In fact it acts as ozone does, when liberated, for example, from hydrogen peroxide. But Pflüger has shown that when blood is poured on filter paper in the way just described, decomposition of the hæmoglobin almost instantly occurs, and it is the products of decomposition which occasion the reaction.

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[1] *Die Blutkrystalle*, p. 134.

[2] Measured at 0° C. and 1 metre pressure; equivalent to 1·67 c.c. measured at 0° C. and 760 millimetres pressure.

[3] Hüfner, *Zeit. physiol. Chem.* i. 317.

We have still the spectroscopic characters of oxyhæmoglobin to consider.

The various forms of spectroscope have been already described (p. 47). It will be sufficient here to repeat that the spectroscope is an instrument which enables us to tell the colour of a solution or transparent substance more accurately than we can with the unaided vision. White light passed through the coloured substance and then through a prism no longer gives a continuous spectrum, but certain parts of it are absorbed, hence the appearance of dark shadows or absorption bands in various parts of the spectrum. These bands remain constant in position for the same substance, and thus furnish us with a delicate test for that substance. We speak of the position of the absorption bands, either by their neighbourhood to certain of the black lines (Fraunhofer's lines) of the solar spectrum; or more accurately still by measuring their position in wave-lengths. The sign λ denotes wave-length; in absorption spectra, the edges of the bands are sometimes so ill defined, and vary in position with the concentration of the liquid, that more often the position of the centre of the band rather than that of its edges is given. λ 500 means a wave-length equal to 500 millionths of a millimetre.

The two next figures illustrate a method of representing absorption spectra diagrammatically. The solution was examined in a layer one centimetre thick. The base line has on it at the proper distances the chief Fraunhofer lines, and along the right hand edges are percentages of the amount of oxyhæmoglobin present in I, of reduced hæmoglobin in II. The width of the shadings at each level represents the position and amount of absorption corresponding to the percentages.

The characteristic spectrum of oxyhæmoglobin (first observed by Hoppe-Seyler) is seen as it actually appears through the spectroscope in the next figure (fig. 59, spectrum 2). There are two distinct absorption bands between the D and E lines; the one nearest to D (the α band) being narrower, darker, and with better defined edges than the other

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[1] *Pflüger's Archiv*, x. 252.

(the β band). The centre of the α band corresponds to λ 579, of t band to λ 553·8 (Gamgee[1]). As will be seen by looking at fig. solution of oxyhæmoglobin of concentration greater than 0·65 per and less than 0·85 per cent. gives one thick band overlapping b and E, and a stronger solution still, only lets the red light th between the C and D lines.

A solution which gives the two characteristic bands must the be a dilute one. But, as before said, we are able with such soluti

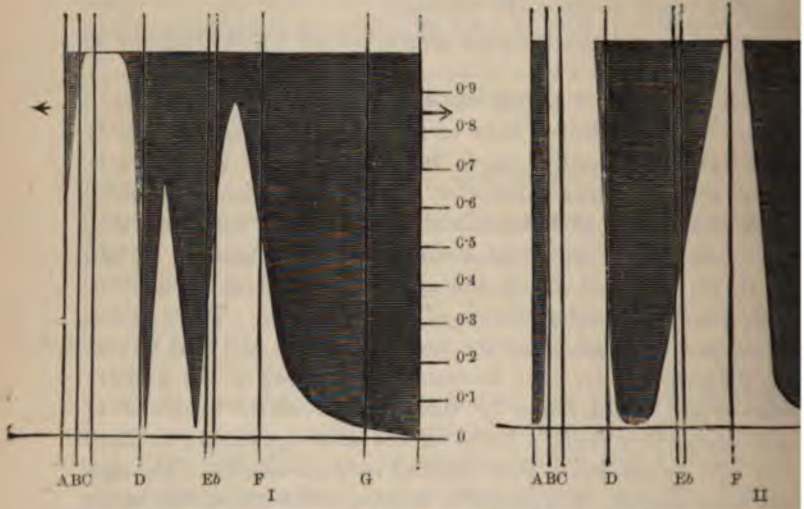

FIG. 58.—Graphic representations of the amount of absorption of light by solution of (I) ox globin, (II) of hæmoglobin, of different strengths. The shading indicates the amount of tion of the spectrum; the figures on the right border express percentages (Rollett).

oxyhæmoglobin, or defibrinated blood will do equally well, to i the reduction of oxyhæmoglobin which occurs in the body. Thi first pointed out by Professor Stokes,[2] who employed for the pu the reducing agent now known as Stokes's reagent.

The following are the means by which we can displace the respir oxygen in a solution of oxyhæmoglobin :—

(1) By boiling it in the Torricellian vacuum of a mercurial air

(2) By passing through the solution a neutral gas such as nitr hydrogen, or carbonic acid.

(3) By the use of reducing agents.

(*a*) Stokes's reagent: a solution of ferrous sulphate, to wh

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[1] Gamgee, *Physiological Chemistry*. Where other wave-lengths are given quently, they are taken either from Gamgee's measurements, or from those m MacMunn, and published in McKendrick's *Physiology*.

[2] Stokes, *Proc. Roy. Soc.* xiii. 357.

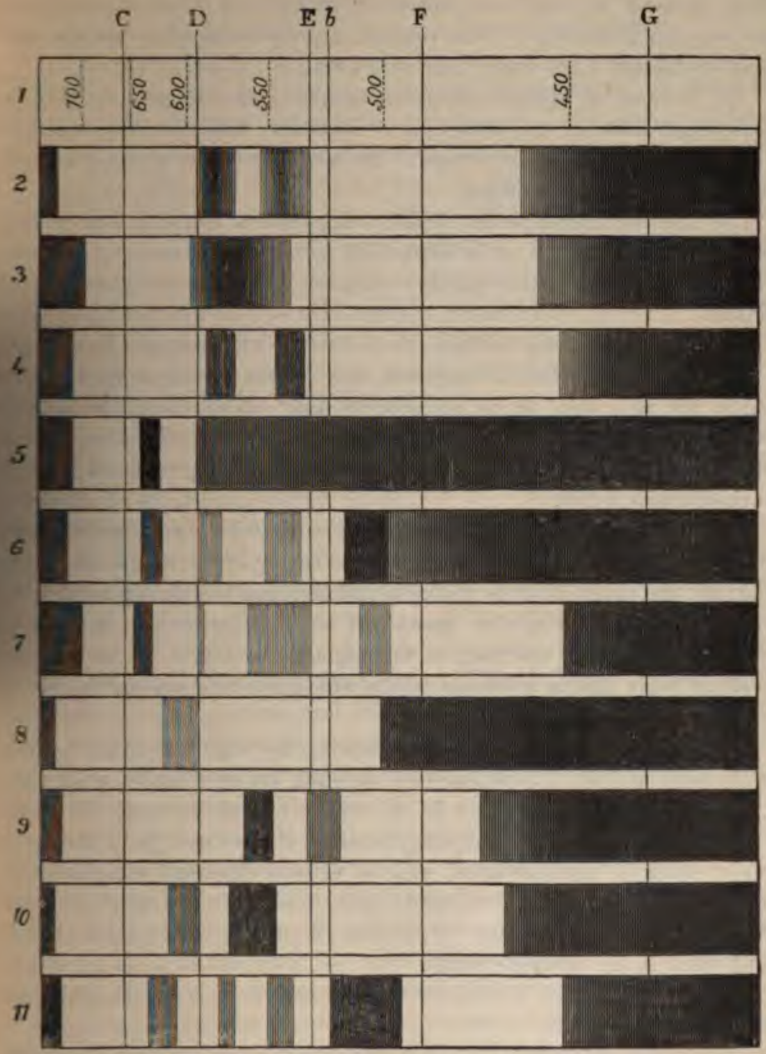

FIG. 59.—1, Solar spectrum. 2, Spectrum of oxyhæmoglobin (0·37 p.c. solution). First band, λ 589–564; second band, λ 555–517. 3, Spectrum of hæmoglobin. Band, λ 597–535. 4, Spectrum of CO-hæmoglobin. First band, λ 583-564; second band, λ 547–521. 5, Spectrum of methæmoglobin (concentrated solution). 6, Spectrum of methæmoglobin (dilute solution). First band, λ 647–622; second band, λ 587–571; third band, λ 552–532; fourth band, λ 514–490. 7, Spectrum of acid hæmatin (ethereal solution). First band, λ 656–615; second band, λ 597–577; third band, λ 557–529; fourth band, λ 517–488. 8, Spectrum of alkaline hæmatin. Band from λ 630–581. 9, Spectrum of hæmochromogen (reduced hæmatin). First band, λ 569–542; second band, λ 535–504. 10, Spectrum of acid hæmatoporphyrin. First band, λ 607–593; second band, λ 585–536. 11, Spectrum of alkaline hæmatoporphyrin. First band, λ 633–612; second band, λ 589–564; third band, λ 549–529; fourth band, λ 518–488. The above measurements (after MacMunn) are in millionths of a millimetre. The liquid was examined in a layer one centimetre thick. The edges of ill-defined bands vary a good deal with the concentration of the solutions.

little tartaric or citric acid has been added, and then ammonia till the reaction is alkaline. This reagent rapidly darkens in the air, and must be freshly made every time it is used.

(*b*) Instead of ferrous sulphate, stannous chloride may be used in the preparation of the foregoing. This has the advantage of not darkening, as it absorbs oxygen. It however must also be always freshly prepared before using.

(*c*) Ammonium sulphide. This on the whole is the most convenient reagent to use, though it is somewhat slower in its action than the two preceding; a little gentle warmth will however hasten its action.

Using any of these methods the colour of oxyhæmoglobin changes to the purplish tint of hæmoglobin, and by the spectroscope the two bands are now seen to be replaced by one, called the γ band; this band is not so well defined as either the α or the β band. Its position between the D and E lines is denoted in fig. 59 (spectrum 3); it is darkest about λ 550.

On dilution the band fades rapidly, so that in a solution of such concentration that both bands of oxyhæmoglobin would be quite distinct, the single band of reduced hæmoglobin has disappeared from view. The oxyhæmoglobin bands can be distinguished in a solution which contains only one part of the pigment to 10,000 of water, and even in more dilute solutions which are apparently colourless, the α band is still visible.

On passing oxygen through a solution of hæmoglobin, or on shaking it up with the air, oxyhæmoglobin showing its two bands, reappears.

2. *Methæmoglobin.*—This is a compound of hæmoglobin with oxygen which can be produced artificially; it also occurs in the body under certain circumstances, e.g. in certain diseased conditions it occurs in the urine (*see* Hæmoglobinuria), and after the administration of large doses of potassium or sodium chlorate it occurs in the blood, and death is the ultimate result.

It may be derived artificially from a solution of oxyhæmoglobin in the following ways:—

(*a*) When a solution of oxyhæmoglobin is exposed to the air in shallow layers for some time, it becomes acid in reaction, brown in colour, and exhibits the characteristic spectrum of methæmoglobin.

(*b*) On the addition of various oxidising agents the same occurs potassium permanganate, potassium ferricyanide, nitrite of potassium nitrite of amyl,[1] &c., act in this way. Hence the view originall advanced by Sorby,[1] that methæmoglobin is more highly oxygenated than oxyhæmoglobin; that it is in fact a per-oxyhæmoglobin.

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[1] Hayem, *Compt. rend.* cii. 698, gives a long list of reagents that act in this way.

(*c*) Methæmoglobin may however be prepared by removing part of the oxygen of oxyhæmoglobin by means of the mercurial air pump, or by means of palladium saturated with hydrogen. Hoppe-Seyler,[2] who describes the above methods, therefore regards methæmoglobin as a sub-oxyhæmoglobin. Whichever view was held as to its constitution, it was admitted by all that the oxygen of methæmoglobin is more firmly combined than that of oxyhæmoglobin. Still it can be removed by reducing agents. The oxygen is however not removable by the air pump, nor by a stream of a neutral gas like hydrogen. On adding ammonium sulphide to a solution of methæmoglobin, the first change is to oxyhæmoglobin, and then to reduced hæmoglobin; these changes can be watched with the spectroscope.

More recently, however, Hüfner and Külz[3] have advanced a third theory concerning the constitution of methæmoglobin, and that is that it contains the same amount of oxygen as oxyhæmoglobin, only in a closer state of combination. They are able to make this assertion from actual analyses;[4] and these analyses were possible, inasmuch as they succeeded in obtaining pure methæmoglobin in a crystalline form. The method of obtaining these crystals is as follows:[5]—Three or four cubic centimetres of a concentrated solution of ferricyanide of potassium are added to a litre of concentrated solution of hæmoglobin. A quarter of a litre of alcohol is added, and the mixture frozen. After one or two days' exposure to this low temperature, abundant crystals of a brown colour, which give the absorption spectrum of methæmoglobin, are deposited. They were obtained in this way from the hæmoglobin of the dog, pig, and horse, and their form is the same as that of the oxyhæmoglobin crystals of the same animals, i.e. rhombic prisms. Gamgee[6] had prepared these crystals from dog's blood many years previously, but their true nature was not at that time recognised. His method was much the same as Hüfner's, the chief difference being that the nitrite of potassium or amyl was employed instead of ferricyanide of potassium.

Jäderholm[7] has also obtained these crystals from dog's blood by the ferricyanide method, and confirms Hüfner's statement that they are rhombic prisms. He also figures some crystals of methæmoglobin obtained by Hammarsten from the horse by the same method, which were regular six-sided plates, and showed no double refraction if lying flat; they therefore presumably belonged to the hexagonal system; they were more insoluble in water than the crystals of dog's methæmoglobin.

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[1] Sorby, *Quart. J. Mic. Science*, 1870, p. 400.

[2] Hoppe-Seyler, *Zeit. physiol. Chem.* ii. 150.

[3] *Zeit. physiol. Chemie*, vii.

[4] Hüfner and Külz employed the spectrophotometric method largely in their work.

[5] G. Hüfner, 'Ueber krystallinisches Methämoglobin vom Hunde,' *Zeit. physiol. Chem.* viii. 366.

[6] A. Gamgee, 'The action of Nitrites on Blood,' *Philos. Trans.* 1868, p. 589, *et seq.*

[7] *Zeitsch. für Biol.* xx. 419. Jäderholm now agrees with Hüfner and Külz with regard to the composition of methæmoglobin.

When one wishes, however, to obtain a small quantity of crystals for microscopic examination, the following simple method may be employed.[1] A few c.c. of the defibrinated blood of an animal (rat, guinea-pig, or squirrel) are taken, and an equal number of drops of nitrite of amyl added. The mixture is vigorously shaken for a minute or two. The colour changes to the dark chocolate tint of methæmoglobin, and spectroscopic observation shows the typical absorption bands of that compound. A drop of this liquid is then placed[2] on a slide and covered; in a few minutes crystals form, which observation with the spectroscope shows to be composed of methæmoglobin. The edges of the cover-glass may then be sealed, and the crystals keep unchanged for several months.

The crystals obtained from guinea-pig's blood by this process are tetrahedra, which differ only in colour and spectroscopic appearances from those of oxyhæmoglobin from the same animal.

The crystals obtained from squirrel's blood are perfectly regular hexagonal plates, which remains dark between crossed nicols.

The crystals obtained from rat's blood are also perfectly regular hexagonal plates,[3] which remain dark between crossed nicols, and which consequently are precisely similar to those of squirrel's methæmoglobin. This remarkable fact helps to show that the difference between the oxyhæmoglobin of these two animals cannot be a very deep or essential one.

The spectrum of methæmoglobin shows three absorption bands, one in the red about half way between the C and D lines, and two others between the D and E lines which resemble in position those of oxyhæmoglobin, but on careful measurement are found to be different.[4] A fourth indistinct band in the blue has also been described (*see* fig. 59, spectra 5 and 6). On adding ammonia to a solution of methæmoglobin, the first two bands shift a little towards the violet end of the spectrum;

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[1] Halliburton, *Quart. Journ. Mic. Science*, xxviii. 201.

[2] This must be done immediately after the formation of the chocolate-coloured liquid, as in about a quarter of an hour the whole liquid sets into a gelatinous mass of the same colour, from which no crystals are obtainable.

[3] A few triangles and forms intermediate between triangles and regular hexagons are also found.

[4] Araki considers that these bands are due to admixture with oxyhæmoglobin (*Zeit. physiol. Chem.* xiv. 405).

this spectrum is sometimes spoken of as that of alkaline methæmoglobin.

3. *Carbonic oxide hæmoglobin.*—This may be readily prepared by passing a stream of carbonic oxide gas[1] through blood, or through a solution of oxyhæmoglobin. Its colour is a peculiar cherry-red. Its absorption spectrum (*see* fig. 59, 4) is very much like that of oxyhæmoglobin, but the two bands are slightly nearer the violet end of the spectrum; the centre of the α band being λ 572, of the β band λ 534 to 538 according to concentration (Gamgee).

CO-hæmoglobin forms crystals like those of oxyhæmoglobin; it is remarkable for its stability; it is not affected by reducing agents like ammonium sulphide, and the carbonic oxide gas can only be driven off by passing through it for a long time a stream of air or of a neutral gas, or by a stream of nitric oxide gas which replaces the carbonic oxide and forms nitric oxide hæmoglobin. CO-hæmoglobin also resists putrefaction for a long time (Hoppe-Seyler[2]).

Carbonic oxide is given off during the imperfect combustion of carbon, such as occurs in charcoal stoves; this acts as a powerful poison by combining with the hæmoglobin of the blood, and thus interfering with normal respiratory processes. The colour of the blood and its resistance to reducing agents in such cases are characteristic. Hoppe-Seyler has, however, introduced another test which has been modified by Salkowski[3] as follows: The blood in question is diluted twenty times, and to some of this in a test-tube an equal volume of aqueous soda of specific gravity 1·34 is added. In a few seconds CO blood becomes whitish, then red; on standing red flocculi separate and finally rise to the surface of a faintly rose-coloured liquid. In normal blood all that is produced by the addition of the alkali is a dirty-brown colouration (hæmatin). Working under Salkowski's direction Katayama[4] has discovered a new test which may be briefly stated as follows: The addition of acetic acid and ammonium sulphide (with sulphur in solution) to normal blood produces a greenish-grey or reddish green-grey colour; to CO blood, a beautiful clear rose-red is produced. This solution shows a spectrum which is a double spectrum, indicating that there is in solution CO-hæmoglobin and sulphur-methæmoglobin (Hoppe-Seyler[5]), viz. one band between C and D, and two others between D and E. The spectrum shown by normal blood after the addition of these reagents is also a double spectrum, viz. of sulphur methæmoglobin and reduced hæmoglobin. In other words, in the case of CO-hæmoglobin the colour of that compound completely masks the olive-green tint of sulphur-methæmoglobin, which spectroscopic observation shows to be present.

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[1] The gas may be generated by adding sulphuric acid to oxalic acid or formic acid in a retort, and then applying heat.

[2] Hoppe-Seyler, *Zeit. physiol. Chem.* ii. 131.

[3] E. Salkowski, *Ibid.* xii. 227.

[4] Katayama, *Virchow's Archiv*, 1888, vol. cxiv. p. 53. References will be found in this paper to other tests which have been proposed for CO-hæmoglobin.

[5] *Physiol. Chem.* p. 388. The composition of sulphur methæmoglobin is not known.

4. *Nitric oxide hæmoglobin.*—When ammonia is added to blood, and then a stream of nitric oxide passed through it, this compound is formed (Hermann [1]); it may be obtained in a crystalline form isomorphous with oxy- and CO-hæmoglobin; it also has a similar spectrum. It is even more stable than CO-hæmoglobin.

Other compounds of hæmoglobin have been described: one with acetylene (C_2H_2), another with hydrocyanic acid (Hoppe-Seyler).[2] Dr. Gamgee[3] has, however, pointed out the unsatisfactory nature of the evidence upon which the existence of such compounds rests.

Recently C. Bohr has advanced the theory that hæmoglobin forms a compound with carbonic acid. The importance of this discovery, if confirmed, is very great, and the question will be discussed in the chapter on Respiration (Chapter XIX).

Estimation of Hæmoglobin

The following methods may be adopted for the quantitative estimation of hæmoglobin :—

1. By the amount of iron in the ash.
2. By colorimetric methods.
3. By spectrophotometric methods.

1. A weighed quantity of blood or substance containing hæmoglobin is evaporated to dryness, the residue is carefully incinerated at a dull red heat, the ash exhausted with hydrochloric acid to obtain ferric chloride. This is reduced by the action of metallic zinc to ferrous chloride, and the amount of iron in this determined volumetrically with a standard solution of potassium permanganate (*see* also p. 25). Dry hæmoglobin contains 0·42 per cent. of iron. If m=percentage amount of iron in the specimen under examination, the percentage of hæmoglobin in that specimen $=\frac{100\,m}{0{\cdot}42}$.

2. Standard solutions of known strength are prepared from crystals

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It is formed by passing a stream of sulphuretted hydrogen through a solution of oxyhæmoglobin. The greenish tint which appears on the surface of corpses a few days after death is due to the development of sulphuretted hydrogen, and the consequent formation of sulphur-methæmoglobin. *See* also Araki (*Zeit. physiol. Chem.* xiv. 412).

[1] Hermann, *Reichert und Du Bois Reymond's Archiv*, 1865, p. 469.

[2] Hoppe-Seyler, *Med. Chem. Untersuch.* Heft ii. p. 207.

[3] Gamgee, *Physiol. Chemistry*, pp. 107–8.

of oxyhæmoglobin. The blood to be investigated is diluted with water until the colour of the standard solution is reached. Knowing the amount of blood and the amount of dilution, the percentage of hæmoglobin is easily calculated (Hoppe-Seyler).

The tint of the solutions must be ascertained by examining them in vessels with parallel sides and of the same width. A hæmatinometer, as such a vessel is called, is usually constructed so that the sides are 1 centimetre apart. Rajewsky[1] and Malassez[2] recommend the standard solution to be made up of picrocarminate of ammonia, the tint of which corresponds to that of an oxyhæmoglobin solution of known strength.

In Fleischl's hæmometer a wedge of red-tinted glass forms the standard of comparison; the wedge is arranged to slide under a hole in a brass plate, the thickness of the glass under observation can thus be varied and adjusted so as to give a red tint equal to that of the blood under examination, which is always diluted to a certain fixed extent.

Gowers' hæmoglobinometer, like Fleischl's instrument, is designed for clinical use. The apparatus consists of two glass tubes, C and D, of the same size. D contains glycerine jelly tinted with carmine to a standard colour, viz. that of normal blood diluted 100 times with distilled water. The finger is pricked and 20 cubic millimetres of blood are measured out by the capillary pipette, B. This is blown out into the tube C, and diluted with distilled water, added drop by drop from the pipette stopper of the bottle, A, until the tint of the diluted blood reaches the standard colour. The tube, C, is graduated into

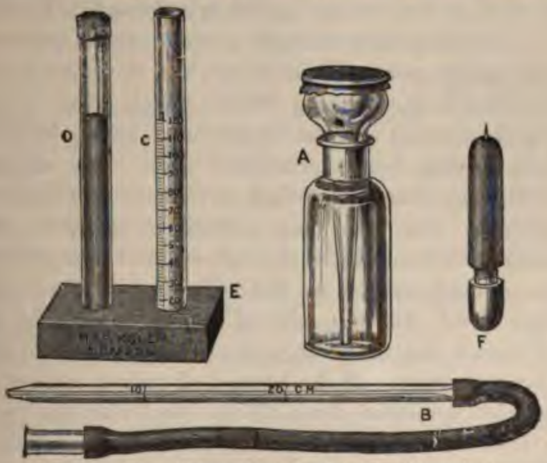

FIG. 60.—Hæmoglobinometer of Dr. Gowers. (Hawksley.)

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[1] Rajewsky, *Pflüger's Archiv*, xii. 70.

[2] Malassez, *Arch. de Physiol.* 1877, p. 1.

100 parts. If the tint of the diluted blood is the same as the standard when the tube is filled up to the graduation 100, the quantity of oxyhæmoglobin in the blood is normal. If it has to be diluted more largely, the oxyhæmoglobin is in excess ; if to a smaller extent, it is less than normal. If the blood has, for instance, to be diluted up to the graduation 50, the amount of hæmoglobin is only half what it ought to be—50 per cent. of the normal, and so for other percentages.

The instrument only yields approximate results, but is extremely useful in clinical observations.[1]

3. The spectrophotometric method for the estimation of coloured solutions has been already described (*see* p. 50).

In connection with the estimation of oxyhæmoglobin it may be added that the region of the spectrum selected for photometric measurements is that of the β band of absorption. This part of the spectrum in the case of oxyhæmoglobin has been found to be that most easily affected by changes in the concentration of the solution through which the light passes.

Glazebrook's spectrophotometer is, in principle, the same as Hūfner's. Light from each of two sources passes first through a Nicol's prism by which it is polarised, then through a direct vision prism ; thus, two adjacent superposed spectra are obtained, and these are observed by an eyepiece in which is an analysing Nicol's prism. This eyepiece can be rotated, and the amount of rotation measured by a pointer attached to the eyepiece moving over a graduated circle. The nicols are then adjusted in the way already described, so that the two spectra appear of equal brightness. One may then proceed to interpose the coloured solution and measure the angles through which it is necessary to rotate the nicols, or, more simply, in the following way, as suggested by Dr. Sheridan Lea.[2] On the path of one beam of light is placed a solution of known concentration, and on the path of the other, one of unknown concentration. If the latter is of greater concentration than the first, it may be diluted down till the effect upon the two spectra is the same ; from the amount of dilution necessary to produce this effect its concentration can be calculated. Or the same effect can be produced by varying the thickness of the layer of the fluid under observation ; this latter plan is found to be perfectly feasible, and is applicable also when the concentration of the unknown solution is less than that of the known ; and Dr. Lea has invented an instrument (absorptiometer) with parallel sides, one of which is movable, and so the thickness of the layer of fluid in it can be varied to known extents. Take as an illustration the following example, which reduces to its simplest elements the method of spectrophotometry. The spectra were first equalised, and a layer of a standard solution of known concentration, C^1, was examined in a layer $\frac{6}{25}$ inch thick. It was found necessary to interpose on the path of the other beam of light a layer $\frac{13}{25}$ inch thick of a solution of unknown concentration, C, in order to make the spectra once more equal. $\frac{C}{C^1}=\frac{13}{6}$, from which equation, C the unknown quantity is easily calculated.

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[1] Gowers, *Lancet*, vol. ii. 1878, p. 822.

[2] Sheridan Lea, *Journ. of Physiol.* v. 239.

Preyer's method is also a spectrophotometric one. A 0·85 per cent. solution of oxyhæmoglobin (thickness of layer being 1 centimetre) is the most concentrated solution which allows the green light of the spectrum to pass through it (*see* fig. 58, sign → ←). Take a known amount of the blood to be investigated and dilute it with water till it just allows the faintest shade of green light to pass through it. If b= volume of blood taken, and w=volume of water added, then the percentage of oxyhæmoglobin in the blood$=\frac{0{\cdot}85\ (w+b)}{b}$.

I append here a table of the results of analysis which I take from Preyer, 'Die Blutkrystalle,' p. 117.

100 grams of healthy human blood contain :—

		Iron	*Hæmoglobin*
Woman	Minimum	0·048 grm.	11·57 grm.
	Maximum	0·057 „	13·69 „
Man	Minimum	0·0508 „	12·09 „
	Average (11 cases)	0·056 „	13·45 „
	Maximum	0·063 „	15·07 „

From data given by Malassez it can be calculated that the amount of hæmoglobin in each human blood corpuscle is approximately 30 billionths of a gramme. Venous and arterial blood contain the same amount of hæmoglobin (Krüger).[1] Fœtal blood is of lower specific gravity than that of adults, and is especially deficient in hæmoglobin.[2]

Determination of the 'Activity of Reduction' of Oxyhæmoglobin.[3]

The *time of reduction* of oxyhæmoglobin is determined by examining the spectrum of the blood under the thumb nail; the first band can be always, the second sometimes, distinguished by the direct vision spectroscope. If a ligature is tied round the phalanx, the bands gradually disappear. The time of reduction is the time they take to disappear, and this in a healthy man at rest averages 70 seconds.

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[1] *Zeit. Biol.* xxvi. 452. Krüger also states that congestion of a part increases the total solids and hæmoglobin in the blood drawn from it; that the blood of the splenic vein is richer, and of the renal vein is poorer, in solids and pigment than arterial blood. Copeman and Sherrington (*Proc. Physiol. Soc.* 1890, p. viii) have, by a different method, arrived at similar conclusions.

[2] Scherrenziss, *Inaug. Diss.* Dorpat, 1888.

[3] Hénocque, *Comptes rendus*, ciii. 817.

The quantity of hæmoglobin in the blood is then determined.[1] A healthy man's blood contains 14 per cent. of oxyhæmoglobin ; from this it is calculated that 0·2 per cent. is reduced per second. This quantity is taken as the unit *activity of reduction*; and the activity in other cases $= \frac{\text{quantity of oxyhæmoglobin}}{\text{time of reduction}} \times 5$.

Hénocque[2] has described the variations in the activity of reduction of oxyhæmoglobin in various diseases, and under the influence of various drugs. In typhoid fever,[3] for instance, periods of high temperature were found to coincide with periods of diminished activity of reduction, and when the temperature was reduced, the activity of reduction increased, and tended to regain its normal value.

Composition of Hæmoglobin

The following is the percentage composition of hæmoglobin as ascertained by various observers.[4]

	Dog			Horse		Guinea-Pig	Squirrel	Goose
	C.Schmidt	Hoppe-Seyler	Jaquet	Kossel	Zinoffsky	Hoppe-Seyler	Hoppe-Seyler	Hoppe-Seyler
C. . .	54·15	53·85	53·91	54·87	51·15	54·12	54·09	54·26
H. . .	7·18	7·32	6·62	6·97	6·76	7·36	7·39	7·10
N. . .	16·33	16·17	15·98	17·31	17·94	16·78	16·09	16·21
O. . .	21·24	21·84	22·62	19·73	23·43	20·68	21·44	20·69
S. . .	0·67	0·39	0·542	0·65	0·391	0·58	0·40	0·54
Fe . .	0·43	0·43	0·333	0·47	0·335	0·48	0·59	0·43
P_2O_5. .	—	—	—	—	—	—	—	0·77

There are thus very considerable discrepancies between the analyses of different observers; we should, however, not be justified in concluding from the results of elementary analyses that there are different varieties of hæmoglobin in different animals ; for if the analyses of hæmoglobin from the same animal be examined there will be found discrepancies equally as great.

A more just conclusion seems to be that the methods we at present adopt are not sufficiently exact to enable us to prepare a pure product.

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[1] Hénocque describes a special form of hæmatoscope for the purpose. Dr. Gowers' instrument would do equally well.

[2] *Comptes rendus*, cvi. 146.

[3] Hénocque and G. Baudouin, *Ibid.* p. 1245.

[4] Hoppe-Seyler's analyses will be found in his *Physiol. Chemie*, p. 377; those of C. Schmidt and Kossel I take from a table in McKendrick's *Physiology*, p. 118; those of Zinoffsky in *Zeit. physiol. Chem.* x. 16, and of Jaquet, who employed Zinoffsky's method, in *Ibid.* xii. 285, xiv. 289. Gschleidlen (*Pflüger's Arch.* xvi. 421) considers that the phosphoric acid of birds' hæmoglobin is due to admixture with nuclein. Jaquet is inclined to consider it as an essential part of the hæmoglobin in itself.

A temperature sufficient to dry the hæmoglobin thoroughly (120° C.) causes, for example, a partial disintegration of this unstable organic compound.[1]

We know of no rational formula for hæmoglobin, and, in view of the discordance of the above analyses, it would seem rash to calculate an empirical one. Preyer's formula is $C_{600}H_{960}N_{154}FeS_3O_{179}$; Hüfner's, $C_{550}H_{852}N_{149}FeS_4O_{149}$; Zinoffsky's, $C_{712}H_{1130}N_{214}FeS_2O_{245}$.

Decomposition of hæmoglobin.—On the addition of acid or alkali to a solution of oxyhæmoglobin, the colour changes to brown; this is due to the decomposition of the hæmoglobin into a proteid, called *Globin*, and a pigment which contains all the iron, called *Hæmatin*. Considerable discussion has taken place on the question as to whether these two substances are mechanically mixed together, or whether they are chemically combined.

It was Lehmann who first brought forward the supposition that hæmoglobin is not a chemical unit, but consists of hæmatin merely mixed with a crystallisable proteid. A seeming confirmation of this theory has been more recently advanced by Struve,[2] who found that by means of alcoholic ammonia hæmatin can be extracted from the crystals, leaving them colourless. Against this, however, it must be pointed out that alcoholic ammonia is a strong reagent, and is able to effect more than a separation of two substances mechanically mixed; even alcohol by itself produces changes in hæmoglobin; the parahæmoglobin crystals of Nencki and Sieber[3] have been shown by Hoppe-Seyler[4] to be a mere coagulation product of oxyhæmoglobin brought about by the action of alcohol; these crystals do not show double refraction—that is, they have not the constitutions of true crystals, but are merely proteid masses which, when coagulated, retain the crystalline shape they had previously.

Another ground upon which some hold that hæmoglobin is not a chemical unit is the conflicting results of analysis, especially with regard to the quantity of sulphur present. Zinoffsky has, however, pointed out that this is due to bad methods of preparation of the hæmoglobin, and by his method he was able to prepare a number of specimens of hæmoglobin in which the analysis gave concordant results. Zinoffsky holds the view, which is now very general, that oxyhæmoglobin is a chemical unit; he also states that its molecule yields on decomposition 1 molecule of hæmatin with 34 atoms of carbon, and 2 molecules of globin each with 1 atom of sulphur and 339 of carbon.

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[1] I have found that, even if the hæmoglobin be dried in a Torricellian vacuum, although a temperature of 40° C. is sufficiently high to drive off all water of crystallisation, it is also sufficient to cause the formation of hæmatin and an insoluble proteid.

[2] Struve, *Zeit. prakt. Chem.* 1884.

[3] Nencki and Sieber, *Arch. experim. Path. u. Pharmakol.* xx. 325.

[4] Hoppe-Seyler, *Zeitsch. physiol. Chem.* x. 331.

Globin.—This proteid is derived from the decomposition of hæmoglobin ; this occurs under the influence of heat, when globin is converted into a heat-coagulum ; by the influence of acids or alkalis, when it is converted into acid and alkali-albumin respectively. In all three cases hæmatin is simultaneously liberated. On heating a solution of oxyhæmoglobin a heat-coagulum forms at 68°–70° C. The flocculi are of a brownish colour, as they carry down with them some of the hæmatin. Under the influence of acid or alkali both hæmatin and the proteid go into solution.

The proteid is a globulin ; it is precipitated from its solutions by saturating them with magnesium sulphate or sodium chloride. Gamgee[1] agrees with Kühne in considering that globin which was first described by Preyer is probably a mixture of proteids.

Hæmatin is a brownish pigment which exhibits different absorption spectra, according as to whether it is dissolved in an acid or alkaline medium. It is insoluble in water, alcohol, and ether, easily soluble in caustic alkalis and in alcohol acidulated with sulphuric acid.

For spectroscopic examination *alkaline hæmatin* may be prepared in the following ways :—

1. Add some strong potash or soda to a solution of oxyhæmoglobin or to some diluted defibrinated blood. The rate at which the decomposition takes place varies in different animals ; the same is also the case with acids (*see* p. 269).

2. Rectified spirit and ammonia, or rectified spirit and soda, may be added to diluted blood, or to a solution of oxyhæmoglobin, and the mixture filtered ; the filtrate shows the spectrum of alkaline hæmatin.

3. Pure hæmatin may be dissolved in caustic alkali. Alkaline hæmatin shows one ill-defined absorption band overlapping D, and extending some distance towards the red end of the spectrum (*see* fig. 59, spectrum 8).

For spectroscopic observation *acid hæmatin* may be prepared in the following ways :—

1. By adding a little glacial acetic acid to diluted blood or solutions of oxyhæmoglobin.

2. By shaking up the acid liquid just prepared with ether ; the ethereal extract on standing floats above the watery liquid and contains the hæmatin dissolved in it.

3. By dissolving pure hæmatin in acidified spirit. Acid hæmatin

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[1] Gamgee, *Physiol. Chemistry*, p. 113.

is sometimes called hæmatoin, or four-banded hæmatin. It shows four bands—one in the red between the C and D lines, but rather nearer the C line than the somewhat similar band of methæmoglobin; one narrow and faint band over the D line (this is especially faint when the hæmatin is prepared by method 3 above); and two bands in the green (fig. 59, spectrum 7).

Pure hæmatin may be prepared in the following ways:—

1. Crystals of hæmin (hydrochloride of hæmatin) are dissolved in dilute potash; this is neutralised with dilute hydrochloric acid, and hæmatin is precipitated as a flocculent brown precipitate, which is collected, washed with boiling water, and dried (Hoppe-Seyler[1]).

2. Blood clot is extracted with rectified spirit containing pure sulphuric acid (1 in 17); the solution is filtered, diluted with an equal amount of water, and agitated with chloroform. The chloroform dissolves out the hæmatin; it is washed with water to remove the acid, and then the chloroform is evaporated; the hæmatin remains as a dark brown pigment which dries up to a bluish-black powder (MacMunn[2]).

The formula for hæmatin is $C_{34}H_{35}N_4FeO_5$, or, perhaps, twice that, $C_{68}H_{70}N_8Fe_2O_{10}$.

It forms compounds with hydrochloric, hydrobromic, and hydriodic acids.

A compound with potassium cyanide is also described.

Hæmochromogen is a reduction product of hæmatin.

Hæmatoporphyrin, hæmatolin, and hæmatoidin are iron-free products of hæmatin.

These different substances must be taken now one by one.

Hæmochromogen.—This substance was called by Stokes reduced hæmatin. It may be readily prepared for spectroscopic observation by adding a few drops of Stokes's reagent or ammonium sulphide to a solution of alkaline hæmatin.

Hoppe-Seyler prepares it from reduced hæmoglobin in an apparatus from which oxygen is excluded, by the action of alcohol containing sulphuric acid, or caustic potash in solution. This method is far more difficult, and involves the use of special apparatus. Whichever method is adopted, the final result is the same: that is to say, whether we first decompose oxyhæmoglobin, and then add a reducing agent; or whether we first reduce the oxyhæmoglobin, and then decompose it.

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[1] Hoppe-Seyler, *Med. Chem. Untersuch.* Heft iv. p. 523.

[2] MacMunn, *Journ. of Physiol.* vi. 22.

On oxidation hæmochromogen yields hæmatin. Hoppe-Seyler represents the reaction as follows :—

$$\underset{\text{[hæmochromogen]}}{2(C_{34}H_{37}N_4FeO_5)} + O_2 = \underset{\text{[hæmatin]}}{C_{68}H_{70}N_8Fe_2O_{10}} + 2H_2O$$

In an alkaline solution hæmochromogen is of a purplish colour; it shows two well-marked bands, one midway between the D and E lines, and the other rather fainter between the E and b lines (*see* fig. 59, spectrum 9). On vigorously shaking up such a solution with the air, the bands disappear, and the hazy shadow-like absorption band of alkaline hæmatin is seen.

Hoppe-Seyler protests against the name reduced hæmatin being applied to hæmochromogen. Hæmatin is an oxidised product of hæmochromogen, but does not contain so much oxygen as oxyhæmochromogen, which in all probability is the compound that contains the respiratory oxygen in oxyhæmoglobin. Hæmatin can be obtained from oxyhæmoglobin by treating it with acids or alkalis, and during this process oxygen is absorbed, probably uniting with the proteid part of the hæmoglobin molecule.[1] Therefore, although hæmatin is an oxidation product of hæmochromogen, Hoppe-Seyler considers that hæmochromogen is not a reduction product of hæmatin. He also considers it probable that hæmochromogen is a ferro-compound, and hæmatin a ferri-compound.

Linossier[2] has proposed a change in the names of these two substances; he suggests that hæmochromogen should be called hæmatin or reduced hæmatin, and that what has hitherto been called hæmatin should be named oxyhæmatin. But if Hoppe-Seyler's view of the relation between hæmatin and hæmochromogen prove correct, this new nomenclature of Linossier's is, of course, inadmissible.

Hoppe-Seyler[3] has recently succeeded in obtaining hæmochromogen in a crystalline form by heating a solution of hæmoglobin with sodium hydrate in the absence of oxygen. By heating a solution of CO-hæmoglobin with the same reagent, crystals of CO-hæmochromogen are formed. Linossier had previously obtained the carbonic oxide and nitric oxide compounds of hæmochromogen.[4] Hæmochromogen is thus the atomic group in hæmoglobin which unites with the gases. To represent it in another way :—

Hæmoglobin = hæmochromogen + globin (proteid).
Oxyhæmoglobin = oxyhæmochromogen + globin.
CO-hæmoglobin = CO-hæmochromogen + globin.

Hæmin.—This substance is the hydrochloride of hæmatin; it crystallises readily, and so forms an easily applied test for blood. The crystals[5] may be prepared for microscopic examination, by boiling a drop of blood with a drop of glacial acetic acid on a glass slide: on cooling, the crystals which are triclinic plates and prisms of a dark brown colour, often in star-shaped clusters, and with rounded angles (fig. 61), separate out.

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[1] Lebensbaum (*Monats. Chem.* viii. 166) finds that oxyhæmoglobin in 0·1 sulphu acid solution absorbs 1·1 per cent. of its weight of oxygen.

[2] *Comptes rend.* civ. 1296.

[3] *Zeit. physiol. Chem.* xiii. 477.

[4] These show characteristic absorption spectra resembling that of oxyhæmoglobin.

[5] Sometimes called after their discoverer, Teichmann's crystals (*Zeit. f. nat. Me* 1853, vol. iii. p. 375, viii. p. 141). For crystallography *see* Lagorio, *J. Russ. Chem. S* 1885, p. 35.

In the case of an old blood stain, when one wishes to apply this test, it is necessary to add a small crystal of sodium chloride in addition to the glacial acetic acid. Fresh blood contains sufficient sodium chloride in itself.

On a large scale, hæmin may be prepared in the following ways :—1. A solution of hæmatin in alcohol acidified with sulphuric acid is heated with a solution of sodium chloride.[1] 2. Defibrinated blood is mixed with a large excess of dilute sodium chloride (1·5 per cent.) solution; the corpuscles when they have subsided are extracted with ether ; the ethereal extract is evaporated to dryness, and the residue heated with glacial acetic acid (Hoppe-Seyler).

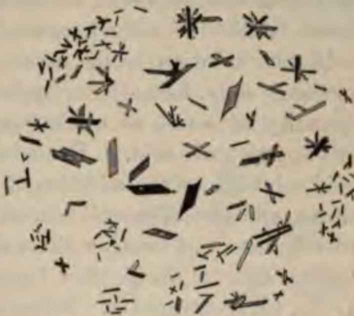

FIG. 61.- Hæmin crystals magnified (Preyer).

Hæmin = hæmatin + 2HCl (Hoppe-Seyler). Hæmin is insoluble in water, ether, chloroform, alcohol, and in cold dilute acetic or hydrochloric acids. It is soluble in an alcoholic solution of potassium carbonate, in caustic alkalis, and in boiling acetic and hydrochloric acids. The crystals are decolourised by alcoholic ammonia (Shalfeeff).[2]

Analogous compounds to hæmin are formed with hydrobromic acid (HBr) and with hydriodic acid (HI). They may be called bromohæmatin and iodohæmatin respectively, while hæmin may be termed chlorohæmatin. The crystalline form and colour of all three compounds are identical (Harris).[3] It has been stated that the preparation of iodohæmatin crystals is a more delicate test for blood stains than that of chlorohæmatin crystals (Bufalini).[4]

Cyan-hæmatin.—'When potassium cyanide is added to an ammoniacal solution of pure hæmatin, or to a solution of oxyhæmoglobin, a broad band extending from D to E is seen on spectroscopic examination. On adding reducing agents, a spectrum with two well-marked absorption bands is obtained. These optical characters are supposed to depend on the production of a compound which has been designated cyan-hæmatin. We are, however, merely acquainted with the spectroscopic characters of the supposed compound.'[1]

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[1] Gamgee, *Physiol. Chem.* p. 117.

[2] Shalfeeff, *Journ. Russ. Chem. Soc.* 1885, p. 203.

[3] V. D. Harris, *Brit. Med. Journ.* vol. ii. 1886, p. 103. *See* also K. Bikfalvi, *Chem. Centr.* 1886, p. 499.

[4] Bufalini, *Arch. Pharm.* (3) xxiii. p. 682. The method consists in heating the aqueous extract of the blood stain with a drop of iodine tincture and a *little* acetic acid on a glass slide. Crystals form in 1–2 minutes. MacMunn obtained a crystalline compound of hæmatin with sulphuric acid, but the chemical constitution of this substance still remains to be worked out (*Journ. Physiol.* vi. 24).

Hæmatoporphyrin.—When hæmatin is heated with fuming hydrochloric acid to 160° C. the iron is removed from it, as a ferrous salt, and iron-free hæmatin or hæmatoporphyrin is formed. The same result is obtained when hæmatin is dissolved in concentrated sulphuric acid, the solution being of a purple-red colour. For spectroscopic purposes only, hæmatoporphyrin may be obtained by adding a small quantity of blood or oxyhæmoglobin solution to a large quantity of strong sulphuric acid.[2] Hæmatoporphyrin may be precipitated from its acid solution by the addition of water. This precipitate is soluble in water, and also in caustic alkalis; the optical properties of the aqueous solution are the same as those of the acid solution, viz. a broad dark band a little to the right of D, and a narrow fainter band to the left of D (fig. 59, spectrum 10). When hæmatoporphyrin is dissolved in caustic alkalis, it appears to undergo some amount of decomposition (Gamgee); the solution has a reddish-brown tint, and the absorption spectrum shows four bands (fig 59, spectrum 11).

Hæmatoporphyrin is interesting as being not only an artificial product of hæmoglobin, but as also occurring in the integument of certain invertebrate animals, viz. starfishes, slugs, the common earth worm, and various sponges (MacMunn).[3] Polyperythrin (Moseley), a pigment of various actiniæ and deep sea polypes, is also probably identical with hæmatoporphyrin. It is also found in the eggshells of some birds. *Urohæmatoporphyrin* is a kind of hæmatoporphyrin found in the urine in certain diseases; it will be fully described under Urine. *Hæmatoporphyroidin* is a decomposition product of hæmatoporphyrin described by le Nobel.[4]

The formula given by Hoppe-Seyler[5] to hæmatoporphyrin is $C_{68}H_{74}N_8O_{12}$; and its formation from hæmatin may be thus represented (*see* further p. 294):—

$$\underset{\text{[hæmatin]}}{C_{68}H_{70}N_8Fe_2O_{10}} + \underset{\text{[sulphuric acid]}}{2H_2SO_4} + O_2 = \underset{\text{[hæmatoporphyrin]}}{C_{68}H_{74}N_8O_{12}} + \underset{\text{[sulphate of iron]}}{2FeSO_4}$$

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[1] Cyan-hæmatin will be found more fully described in Hoppe-Seyler's *Med. Chem. Untersuchungen*, Heft iv. The above short description I have taken from Gamgee's *Physiol. Chem.* p. 115.

[2] Hoppe-Seyler has obtained hæmatoporphyrin by the action of nascent hydrogen, and MacMunn by the action of sodium amalgam on hæmatin.

[3] MacMunn, *Quart. Journ. of Mic. Science*, 1877; *Journal of Physiology*, vols. vii. and viii. It may be added that in certain species of actiniæ a pigment very similar to hæmochromogen, and convertible into hæmatoporphyrin, is found (*Phil. Trans.* 1885).

[4] *Chem. Centralbl.* 1887, p. 538.

[5] Hoppe-Seyler has described another iron-free derivative of hæmatin, to which he gives the provisional name of hæmatolin ($C_{68}H_{78}N_8O_7$). It differs from hæmatoporphyrin in being insoluble in sulphuric acid and caustic alkalis (*Physiol. Chemie*, p. 397).

Hæmatoidin.—In old blood clots, such as occur in the brain after cerebral hæmorrhage, in the interior of aneurisms, and in the corpora lutea of the ovary, small rhombohedral crystals of a brick-red colour are often found, together with an amorphous deposit[1] of the same colour (*see* fig. 62). The name hæmatoidin was given to this substance by Virchow.[2] It is insoluble in water, alcohol, ether, acetic acid, dilute mineral acids and alkalis; soluble in concentrated acids and caustic alkalis.

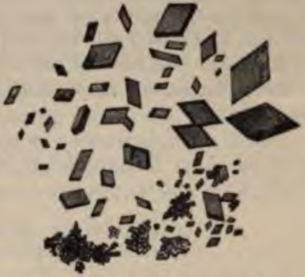

FIG. 62.—Hæmatoidin crystals.

When treated with fuming nitric acid, the crystals give the same colour reaction as the bile pigment does[3] (Gmelin's reaction). Hæmatoidin is undoubtedly a derivative of hæmoglobin, and it is free from iron: so also is bilirubin, the pigment of the bile; and Salkowski[4] found hæmatoidin to be identical chemically with bilirubin. Berzelius found in the gall-bladder crystals of bilirubin exactly similar to those of hæmatoidin. Preyer, however, states that the two substances differ spectroscopically; solutions of bilirubin showing no bands, solutions of hæmatoidin showing one band between b and F, and a weaker one between F and G. Holm[5] obtained similar results. Thudichum[6] has pointed out that Preyer and Holm mistook the lipochrome (lutein) in the cow's ovary for hæmatoidin, and hence they concluded that it was not identical with bilirubin. Neither hæmatoidin nor bilirubin shows bands, but both possess a strong absorptive power for the violet end of the spectrum.

Hæmosiderin is the name given by Neumann[7] to a pigment often occurring in extravasations and thrombi with hæmatoidin, but differing from it in containing iron.

So far, in describing the composition of hæmatin and its derivatives, I have followed Hoppe-Seyler pretty closely. More recently, however, the subject of hæmatin and its allies has been reinvestigated by Nencki and Sieber,[8] and the conclusions at which they have arrived are on many points different from those of Hoppe-Seyler[1]: to certain of these, however, much importance cannot be attached; for instance, until we know a rational formula for hæmatin it matters but little whether in its empirical formula there are a few atoms more or less of any one element. In certain other matters, for instance, in the composition of hæmin, their views demand confirmation; I have, therefore, retained the description of this substance as usually given, and here add a résumé of Nencki and Sieber's work:—

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[1] The amorphous variety was first described by Robin, *Ann. Chem. Pharm.* cxvi. 89. *See* also Städeler, *Ibid.* cxxxii. 328.

[2] *Virchow's Arch. d. pathol. Anat. u. Physiol.* vol. i. (1847), p. 383.

[3] Jaffe, *Arch. f. path. Anat.* xxiii. 192. Hoppe-Seyler, *Ibid.* xxiv. 10.

[4] Salkowski, *Hoppe-Seyler's Med. Chem. Unters.* Heft iii. p. 436.

[5] Holm, *Journ. f. prakt. Chem.* c. 142.

[6] Thudichum, *Proc. Roy. Soc.* xvii. 255. Dr. MacMunn kindly furnished me with this and several of the foregoing references.

[7] *Virchow's Archiv*, cxi. 25.

[8] Nencki and Sieber, *Berichte d. deutsch. chem. Gesellschaft*, xvii. 2267, xviii. 392. *Monatsh. Chem.* ix. 115.

They ascribe to hæmatin the formula $C_{32}H_{32}N_4FeO_4$; and the word hæmin is applied, not to the hydrochloride of hæmatin, but to an anhydride of hæmatin, of which the formula is $C_{32}H_{30}N_4FeO_3$. What is usually called hæmin (chlorohæmatin) they call hæmin-hydrochloride, of which the formula is $C_{32}H_{30}N_4FeO_3.HCl$. This is a crystalline substance, and in its preparation these observers employ amyl alcohol, and a molecule of amyl alcohol of crystallisation is combined with the crystals, the full formula for which would therefore be $C_{32}H_{30}N_4FeO_3.HCl.C_5H_{12}O$. This molecule of amyl alcohol can be driven off by heating the crystals to 130° C.

Hæmatoporphyrin may be obtained by the action of concentrated sulphuric acid on either hæmatin ($C_{32}H_{32}N_4FeO_4$) or hæmin ($C_{32}H_{30}N_4FeO_3$). Its formula is $C_{32}H_{34}N_4O_5$, and its formation may be represented by this equation:—

$$\underset{\text{[hæmatin]}}{C_{32}H_{32}N_4FeO_4} + \underset{\text{[sulphuric acid]}}{H_2SO_4} = \underset{\text{[hæmatoporphyrin]}}{C_{32}H_{32}N_4O_5} + FeSO_4 + H_2O$$

They subsequently found that when hæmin or hæmatin is heated with a saturated solution of hydric bromide in glacial acetic acid it is readily converted into hæmatoporphyrin, which shows differences from that obtained in the usual way. It has the formula $C_{16}H_{18}N_2O_3$. It is insoluble in water and dilute acetic acid, slightly soluble in ether, amyl alcohol, and chloroform, and readily soluble in alcohol, dilute mineral acids, and solutions of the alkalis. It is reddish-brown in colour, is amorphous, and turns brown and becomes more insoluble at 100° C. Its alkaline solution shows the four bands previously described. The hydrochloride ($C_{16}H_{18}N_2O_3.HCl$) crystallises in tufts of needles,[2] the sodium salt ($C_{16}H_{17}NaN_2O_3 + H_2O$) in microscopic prisms.[2] This substance is considered to be pure hæmatoporphyrin, and the substance prepared by the action of sulphuric acid on hæmatin is probably its anhydride ($C_{32}H_{32}N_4O_5 = 2C_{16}H_{18}N_2O_3 - 2H_2O$).

Hæmatoporphyrin has the same empirical formula as bilirubin, which it resembles in many of its properties. The formation of bilirubin from hæmatin is represented by Nencki and Sieber by the equation:—

$$\underset{\text{[hæmatin]}}{C_{32}H_{32}N_4O_4Fe} + 2H_2O = \underset{\text{[bilirubin]}}{C_{32}H_{36}N_4O_6} + Fe$$

When introduced into the living subject hæmatoporphyrin is partly expelled in the urine, but the greater portion is retained, and is probably utilised in the formation of hæmoglobin.

Taking a general survey of the subject, we are able, in spite of contradictory assertions on minor points, to draw a few conclusions. There seems to be a group of iron-free derivatives of hæmatin, and not a single one. Some of these can be produced artificially, such as hæmatoporphyrin with its anhydride, and hæmatolin; certain others occur in the organism of certain lower animals as such (hæmatoporphyrin), and certain others are formed during the normal or abnormal disintegration of hæmoglobin that occurs in the course of the manufacture of bile and urine pigments (bilirubin, urobilin and urohæmatoporphyrin); and lastly one is formed in the disintegration of the blood pigment, that occurs in an old blood clot (hæmatoidin). In spite, however, of differences between these different forms of iron-free hæmatin (in solubilities, optical characters, &c.), one cannot help being more struck with the resemblances between them. It therefore appears possible that we may eventually find we are dealing with a number of isomeric or polymeric substances, for in three of them already (bilirubin, hæmatoporphyrin, and hæmatoidin) the same empirical formula has been described.

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[1] Hoppe-Seyler has discussed the experiments of Nencki and Sieber, and reaffirmed his own views in the *Berichte d. deutsch. chem. Gesellsch.* xviii. 601.

[2] Figured in *Archiv f. exp. Path. u. Pharmakol.* xxiv. plate iv.

TESTS FOR BLOOD

In medico-legal cases it is often necessary to ascertain whether or not a red fluid or stain upon clothing is or is not blood.

The tests to be applied are microscopic, chemical, and spectroscopic.

Microscopic tests.—The corpuscles of the blood should be sought for. If the blood is fairly fresh it is possible to distinguish the human red corpuscles from the red corpuscles of those animals in which they are nucleated, or differ from them greatly in size and shape. Exceedingly careful measurements have shown that there are small but very small variations in the diameter of the human red corpuscles and those of the common mammals, but practically it is not possible to discriminate between them.

Chemical tests.—The old test with tincture of guaiacum and hydrogen peroxide, the blood causing the red tincture to become green, is very untrustworthy, as it is also given by many other organic substances, such as potatoes, certain forms of filtering paper, &c. &c. The only trustworthy chemical test is the formation of hæmin crystals; if one only has a piece of stained clothing to deal with, this is boiled with glacial acetic acid, and a small crystal of sodium chloride on a slide; on cooling the crystals form as already described.

Spectroscopic tests.—If the blood is present in any quantity, the typical bands of oxyhæmoglobin can be readily seen through the spectroscope; these give place to the single band of hæmoglobin on the addition of a reducing agent. One must be prepared, however, in the case of old stains for the presence of methæmoglobin, or of hæmatin.

In such a case, and also when the quantity of hæmoglobin is very small, the most readily obtained spectrum is that of hæmochromogen or reduced hæmatin. The stained fabric is extracted with a small quantity of water; a few drops of a reducing agent (freshly prepared sodium hyposulphite is a good one to use),[1] and then a few drops of concentrated caustic soda solution to decompose the hæmoglobin; the spectrum of hæmochromogen, or at any rate the best marked band of that substance (the one between D and E), then appears; the mean wave-length of this band is λ 557. This band disappears on heating to 50° C. and reappears on cooling; it also disappears when the solution is agitated with the air, but the substance so formed is alkaline hæmatin, which shows only a faint band, too faint to be seen in such weak solutions as we are considering.

In cases where the stain has become insoluble in water, it must be dissolved out with ammonia, and the solution reduced by Stokes's reagent; the typical band of hæmochromogen is then seen. This test is applicable even in cases where no hæmin crystals are obtainable.

In any particular case it is advisable not to rely upon one test only, but to try every available means of detection at one's disposal.

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[1] Recommended by Linossier, *Bull. Soc. Chim.* xlix. 691.

CHAPTER XVI

THE BLOOD IN DISEASE

THE common expression 'The blood is the life' expresses what was till comparatively recent years regarded as true by scientists, namely that the most important of the vital processes take place in that fluid; the chemical changes grouped together under metabolism, we now know occur not in the blood, but in the tissues generally, the blood forming in great measure a means of putting the other tissues into communication with those parts where nutriment is obtained, or excretions discharged.

Not only was this view held of the importance of the blood in the processes of health, but in disease also it was supposed to play an equally leading part. This gave rise to the doctrine of disease called humoral pathology; and its exponents were called humoralists; the opponents of this exclusive view of disease arose about the middle of this century, and were dubbed solidists or anti-humoralists. The great stimulus in starting the opposition to humoral doctrines was no doubt Schwann's great discovery of the important part that the animal cell plays both in health and disease, and the anti-humoralists regarded the life of the organism as the sum of the life of all the constituent cells of its various organs; similarly too, disordered conditions of the blood were considered to be either secondary to changes in the other tissues, or if primary, that they produced their results by the effects such changes had on the other tissues.

In the present day, half a century since the promulgation of the cell theory, physicians are now better able to weigh these two counter-doctrines, than was possible in the first flush of a new and brilliant discovery, and their relative importance can be now more fairly estimated. It is now well recognised that all diseases are not morbid conditions of the blood, and that unhealthy blood is often the result of disorders elsewhere; in the same way, bleeding is not resorted to as a panacea for every ill; but on the other hand it is also perfectly well recognised that in certain diseases the defect is either in the blood itself, or in the blood-forming organs; and that in many diseases we have very distinct evidence of the presence of abnormal substances or poisons in the blood itself; gout is an instance of this; but in the early days of anti-humoralism the humoral nature of gout was stoutly denied.

In the following brief description of the various altered blood conditions seen in disease, it will be convenient to take first those conditions in which the primary mischief seems to be in the blood itself, and secondly those in which the morbid state of the blood is part of a general pathological condition, or secondary to changes in other organs.

THE BLOOD IN ANÆMIA

Anæmia is a term which covers a large number of cases in which poorness in one or other, or all the constituents of the blood, is the one constant condition.

It may be the result of numerous and very varying conditions; when the blood-forming tissues are at fault, it may be considered a primary disease of the blood itself; but there are a large number of other cases in which anæmia is the accompaniment of a general state of debility or malnutrition, or secondary to chronic affections of other organs.

It may be the result of excessive hæmorrhage; the blood then becomes rapidly diluted with lymph, and thus the corpuscles are less numerous than normal until fresh ones are formed to replace those that were lost; the total of solids of the plasma is also diminished, as the lymph is more watery than normal plasma.

Not only hæmorrhage, but other discharges also, produce an anæmic condition, such as the discharges from abscesses, excessive and chronic diarrhœa, discharge of albuminous urine in Bright's disease, and so forth.

On the other hand, the intake of nutriment may be insufficient, and thus the whole body, including the blood, may be wasted. Insufficient food, unsanitary conditions, i.e. insufficient air, light, and exercise, are all potent causes of anæmia. The wasted condition may not however be the result of bad hygiene; malnutrition may arise from an inability to take food, owing to obstruction in the œsophagus, to disease such as catarrh, or the more serious condition, cancer of the stomach, and to many other morbid states of the alimentary organs.

In chronic diseases generally, in chronic lead or mercurial poisoning, there is also associated a marked anæmic condition.

Lastly we have those primary conditions of anæmia which are called chlorosis, pernicious anæmia, and leucocythæmia.

In anæmia generally it may be stated that the most marked effect is seen in a diminution of the number, size, and colour of the red corpuscles, often an increase actual as well as relative of the white corpuscles, and an increase of water and diminution of the solids of the plasma. The changes in the corpuscles are investigated clinically by the microscope, the hæmocytometer, and hæmoglobinometer ; while the changes in the plasma require the more complicated methods of analysis, all of which have been described in the foregoing chapter.

In *chronic anæmia* the red corpuscles may be diminished to $\frac{1}{4}$, or in extreme cases to $\frac{1}{6}$, of the normal amount. In cases of moderate intensity the amount of hæmoglobin varies between $\frac{1}{2}$ and $\frac{1}{4}$, and in extreme cases to $\frac{1}{8}$ of the normal amount. In all chronic cases the mean diameter of the red corpuscles falls to 7 μ or even to 6 μ ; there are also an unusually large number of small red corpuscles (diameter $2{\cdot}2-6$ μ), and almost as frequently a certain number of unusually large corpuscles (diameter $10-12$ μ). Some of the smaller corpuscles seem to have less consistency than normal, and assume modified, often oval shapes.[1] In anæmic blood the clot shows usually a buffy coat ; this does not seem to be due to the coagulation being very slow, but rather to the subsidence of the red corpuscles being very quick, on account of the low specific gravity of the plasma.

Chlorosis is a condition of anæmia which occurs almost exclusively in young women, and is associated with disorder of the menstrual function. There is intense anæmia, falling most especially on the red corpuscles which are few and pale ; this produces a peculiar greenish pallor of the skin, from which the disease derives its name. Chlorosis also is that form of anæmia in which the administration of iron causes the best effects. Often after only a few days the corpuscles are increased, a red colour has returned to the cheeks, and all the other troubles such as palpitation, breathlessness, &c., due to an insufficient amount of hæmoglobin, disappear. The following analyses give the condition of the blood before and after the medicinal use of iron in two cases (Andral and Gavarret[2]).

	CASE 1		CASE 2	
	Before Iron	*After Iron*	*Before Iron*	*After Iron*
Water in 1,000 parts	866·7	818·5	852·8	831·5
Fibrin	3·0	2·5	3·5	3·3
Blood corpuscles .	46·4	95·7	49·7	64·3
Solid residue of serum	83·9	83·3	94·0	100·9

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[1] Many of the above facts regarding the corpuscles in anæmia I have taken from the admirable epitome of Hayem's work given in Gamgee's *Physiol. Chem.* p. 148. Hayem, *Recherches sur l'anatomie normale et pathologique du sang*, Paris, 1878. *Du sang*, Paris, 1889.

[2] Andral and Gavarret, *Annales de chimie et de physique*, lxxv. 225.

Considerable doubt has, however, arisen as to whether the iron administered is actually absorbed; there are many who believe that the iron is absorbed; on the opposite side, Hamburger among others considers that little or none of the medicinal preparations of iron is absorbed from the alimentary canal, but that iron is absorbed only in the form of organic compounds, such as are formed in the synthetic processes of plant and animal life. The quantity of iron in the whole body is only three grammes, and this quantity is taken many times over during treatment. Bunge[1] explains the usefulness of iron in chlorosis by its forming iron sulphide in the intestines, removing in this way excess of sulphur from the body; in chlorosis there are excessive fermentation processes in the alimentary canal, and large quantities of sulphuretted hydrogen are formed, which destroy the organic compounds of iron that form hæmoglobin (hæmatogen); the administration of iron prevents this destruction of hæmatogen. Landwehr[2] points out that such a theory, however, does not explain the limitation of the disease to the female sex, and the period of early adolescence. He regards the disease as one produced by an excessive development at this period, of substances containing animal gum[3] necessary for the nourishment of the embryo, and which act injuriously on the hæmoglobin molecule. If this is so, chlorotic people should take little or no carbohydrate food. Landwehr further considers that iron precipitates the gum in the alimentary canal as a jelly-like coagulum, and thus excess of gum leaves the body with the fæces.

Progressive pernicious anæmia.—By some this disease has been regarded as simply an advanced form of ordinary anæmia, but its rapid development, and its usually fatal termination, as well as certain other peculiar symptoms (attacks of pyrexia, liability to retinal hæmorrhage, &c.), place it on a different footing from ordinary anæmia, and most clinical observers recognise it as a distinct disease.

The disease was first described by Drs. Wilks and Addison, but since then numerous observers have added greatly to our knowledge of its symptoms and pathology. We have here, however, only to deal with the changes in the blood, and the probable cause of those changes.

The changes in the blood have been very thoroughly investigated by Eichhorst,[4] and may be summarised as follows:—

1. The coloured corpuscles are diminished in number, and in the amount of hæmoglobin they contain. There is a great increase in the

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[1] Bunge, *Zeit. physiol. Chem.* ix. 49.

[2] Landwehr, *Pflüger's Archiv*, xl. 21.

[3] The question of animal gum and its relation to mucin and similar bodies will be found discussed in detail under the heading Connective Tissues.

[4] *Die progressive perniziose Anämie*, Leipzig, 1878.

number of the small red corpuscles, many of which are mis-shapen, and many of which are globular and not discoid. The non-discoid corpuscles are not, however, constantly present.[1] Occasionally nucleated coloured corpuscles have been observed (Byrom Bramwell).[2]

2. The colourless corpuscles are also few in number.

3. The blood when shed coagulates with difficulty.

The question arises, is this disease due to diminished formation of the elements of the blood, or to increased destruction of the same? In those cases where nucleated red corpuscles have been found, it has been assumed that the red marrow is diseased, as in certain forms of leucocythæmia, which will be presently mentioned. But the majority of cases show no disease of the red marrow, and the usual view held is that pernicious anæmia is due to excessive destruction of the cellular elements of the blood. Recent researches by Dr. W. Hunter[3] and Dr. Mott[4] on the pathology of the disease fully confirms this theory; I quote briefly Hunter's conclusions with regard to the nature of the blood destruction. It is not simply a dissolution of the red corpuscles in the general circulation, such as occurs periodically in paroxysmal hæmoglobinuria, or may be artificially induced by the injection of distilled water or pyrogallic acid into the circulation. Hæmoglobinuria is always absent in pernicious anæmia. In this relation the condition of the liver is of the greatest importance. The condition of the liver is as follows: (1) It is exceedingly rich in iron; (2) there is excess of pigment within the liver cells; and (3) there is fatty degeneration in the central third of each lobule. A condition closely resembling this, though not so marked, is produced by the drug toluylendiamine. It is therefore assumed that the agent, or agents, which induces the excessive destruction of blood in pernicious anæmia is one whose action on the blood and on the liver cells is the same as that of toluylendiamine. This view is strengthened by the consideration that the form assumed by the hæmoglobin after its liberation from the corpuscles is, in cases of pernicious anæmia, similar to that assumed by it after poisoning by toluylendiamine. After poisoning by this drug numerous small globules of a yellowish colour occur in the urine, and these exactly resemble the globules of pigment found in the convoluted tubules of the kidney in certain cases of pernicious anæmia.[5] The urine contains excess of urobilin (Mott).

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[1] Grainger Stewart, *Brit. Med. Journ.* vol. i. 1876, p. 40.

[2] *Edinburgh Med. Journ.* xxiii. 408.

[3] Hunter *Lancet*, vol. ii. 1888, p. 654. In this paper will also be found reference to researches of others who have worked at the subject.

[4] *Ibid* vol. i. 1889, p. 520; vol. i. 1890, p. 287.

[5] Hunter does not however give any proofs that these yellow globules consist of

With regard to the precise nature of the poison generated, Hunter suggests it may be of a cadaveric nature, absorbed from the alimentary tract. The research is, however, specially valuable in fixing the seat of the disintegration of the corpuscles in the portal circulation, and its important *annexa*, the spleen and liver. (*See* also Liver, Spleen, Urine.)

Hæmoglobinuria.—This is a condition in which the hæmoglobin of the red corpuscles becomes dissolved in blood plasma (hæmoglobinæmia), and passes into the urine, mostly in the condition of methæmoglobin. This condition will be more fully described under Urine.

Leucocythæmia.—The normal proportion of white to red corpuscles in man is about 1 : 350. This proportion is, however, by no means fixed; it varies in different vessels, at different times of the day, with different ages. There are also certain conditions in which the white corpuscles are increased, but still not to such a great extent as to produce the symptoms of what is called leucocythæmia. Thus in many forms of chronic anæmia, the white corpuscles are slightly raised in number absolutely as well as relatively. During pregnancy there is a similar condition, and in many inflammatory affections it occurs also. The term *leucocytosis* is applied to this condition, whereas the word leucocythæmia is not used until the proportion of white to red reaches 1 : 20. In some cases, however, the proportion may be as high as 1 : 6, or even, it is said, 1 : 3; and in these cases when the blood is shed it has the appearance of a mixture of blood and pus.

The disease is usually accompanied with great hypertrophy of the spleen; sometimes with a general hypertrophy of the lymphatic glands throughout the body; it is, however, quite possible to have a very great increase in the size of the lymphatic glands (lymphadenoma) without any leucocythæmia.[1] There are other cases again in which the red marrow is diseased (myelogenic leucocythæmia), and nucleated red corpuscles like those of the embryo are found in the circulation.

It is possible that in certain cases of leucocythæmia and other forms of intense anæmia the affection of the red marrow may be secondary rather than primary. Thus Denys,[2] who has investigated the formation of red corpuscles in pigeons, finds that during simple

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hæmoglobin or are derived from it; they may indeed be often found in perfectly normal kidneys, and give none of the reactions of hæmoglobin.

[1] In one third of the cases of splenic leucocythæmia the lymphatic glands are also enlarged; under the microscope sections of the glands appear normal, there is rarely the increase of the interstitial reticulum that occurs in lymphadenoma. Gowers, *Reynolds's System of Medicine*, vol. v. p. 238–9.

[2] Denys, *La structure de la moelle des os chez les oiseaux. Travaux du laboratoire d'anatomie pathologique de l'université de Louvain.*

inanition the blood-forming structures degenerate, and are replaced by a mucus-like tissue, and that imperfectly formed or embryonic corpuscles make their way into the general circulation.

Not only is the number of white corpuscles increased, but the red are diminished in number.

Elongated, octahedral, colourless crystals have been stated to separate from the blood of leucocythæmic patients after death by several observers (Charcot, Vulpian, Salkowski, Zenker), and different views have been held as to their nature; they have been variously considered to consist of proteid, of mucin, and of the phosphate of a base with the formula C_2H_3N (Schreiner[1]). These crystals are usually spoken of as Charcot's crystals.[2] They are not, however, peculiar to leucocythæmia; they have been found also in cases of simple anæmia, and in the sputum of bronchial asthma (Leyden[3]).

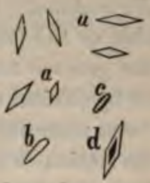

FIG. 63.—Charcot's crystals after Zenker; a few have the angles rounded.

Xanthine and hypoxanthine occur in greater abundance in leucæmic blood than in normal blood; these, no doubt, are derived from the white corpuscles, according to Kossel[4] from the nuclei of white corpuscles. Lactic acid, which has been also described, doubtless owes its origin to the increased number of white corpuscles, which undergo changes resulting in the formation of this acid when the blood is shed. (*See* footnote 1, p. 261.)

Scherer and Gorup-Besanez[5] have described in the blood in this disease a substance which is soluble in hot water, and sets into a jelly when its solution is cooled, in fact, which behaves like gelatin.

Addison's disease.—This is a disease which is associated with great wasting and anæmia; the skin is deeply bronzed, and in typical cases the suprarenal capsules have been found (*post mortem*) to be diseased. The relation between bronzing and suprarenal capsular disease is, however, by no means constant, and by some observers the changes occurring in the neighbouring semilunar ganglia are regarded as more important than those in the suprarenals.

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[1] *Liebig's Annalen*, cxciv. 68.

[2] A full account with references will be found in Gowers's article in *Reynolds's System* just quoted, p. 233.

[3] More recently Meissen (*Berlin. klin. Wochensch.* No. xxii. 1883) has described the same crystals in the expectoration of phthisical and bronchitic patients.

[4] Kossel, *Zeitschr. f. physiol. Chem.* vi. 7–22. *See* also Salomon, *Archiv f. Anat. u. Physiol.* 1876, p. 762.

[5] *Maly's Jahresbericht*, iv. 126.

Nevertheless, it is interesting here to note MacMunn's[1] discovery of hæmochromogen in the medulla of the suprarenal capsules of mammals. This is partially removed by washing out the blood vessels with salt solution. Hence, and owing also to the fact it is elsewhere excretory, the hæmochromogen of the adrenals is probably excretory too. MacMunn further considers that if the adrenals are functionless, as in Addison's disease, the metabolism of hæmoglobin (and of allied pigments, to which he has given the name histohæmatins) is prevented, and the incompletely metabolised pigments circulate in the blood, and lead to staining of the skin and mucous membranes.

Myxœdema.—In a few cases of this disease in man, the red corpuscles, or the hæmoglobin, have been observed to be diminished, but in the greater number of cases, no characteristic changes have been noted by clinical observers, and in the few instances in which the blood has been more fully examined the only noteworthy alteration seen was the formation of a buffy coat on the clot.

In certain animals the disease can be produced experimentally by removal of the thyroid gland, and disease or atrophy of the thyroid gland is the cause of the disease in the human subject also. Some animals (for instance, pig, donkey), however, do not exhibit the typical symptoms of the disease, and in these the blood and serous effusions are perfectly normal. But, on the other hand, in monkeys, which exhibit the disease in a very characteristic manner, and show the swelling of the connective tissue which will be fully described with that tissue, it is found that the blood also exhibits certain marked changes, viz. anæmia, slow coagulation with formation of a buffy coat, and presence of small quantities of mucin,[2] which increase as the disease becomes fully developed. In dogs also, leucocytosis and anæmia follow the extirpation of the thyroid.

Enumeration of the corpuscles in the thyroid vein and artery respectively has shown that there is a distinct surplus in the former vessel. One would, therefore, be inclined to conclude that the gland was concerned in the formation of corpuscles; and that this supposition is in part correct, is confirmed by the existence in nodules throughout the gland of a tissue resembling closely that of the spleen, and in some few cases removal of the gland has been followed by enlargement of the spleen.

The general alterations throughout the whole body seem, however, to point to the function of the gland as concerned not so much in the elaboration of the corpuscles as of certain constituents of the plasma;

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[1] *Proc. Roy. Soc.* xxxix. 248.

[2] Or at least of a substance readily precipitable by acetic acid and insoluble in excess of that reagent.

and the overgrowth of the connective tissues, and the accumulation of an abnormal product in the blood, have led some observers to suppose that the gland is concerned in the separation of mucin from the blood, and then the completion of its metabolism into simpler products.

A full account of myxœdema, clinical and experimental, will be found in the report of a Committee of the Clinical Society, published as an Appendix to Vol. XXI. of the Clinical Society's Transactions.

PATHOLOGICAL CONDITIONS IN WHICH THE COAGULATION OF THE BLOOD IS ABNORMAL

We have already seen (p. 224) that the living vessels exercise a powerful restraining influence upon coagulation; there are, however, certain pathological processes in which the blood may coagulate within the vessels during life; this may be from an alteration in its chemical constitution, as after certain acute specific diseases, such as typhoid fever, or after pregnancy (phlegmasia dolens); the nature of the chemical changes is, however, not known; in some cases the intravascular coagulation is so extensive as to cause death, especially when the blood in the pulmonary vessels is affected; in other cases where recovery ensues, the resolution of the clot is often rapid; vessels which one day can be felt as hard cords beneath the skin may the next day be quite pervious, and no trace of a clot can be felt.[1] In other cases the intravascular coagulation is due to the absorption or experimental injection of poisonous substances into the circulation. These are probably proteid in nature. Thus in a wound in which the discharge is pent up, the absorption of such exudation will in mild cases bring on a rise of temperature causing one form of surgical fever,[2] but in other cases will cause intravascular coagulation. The material which produces these poisonous effects is probably the fibrin-ferment;[3] the normal body has the power of resisting the action of this substance to a very great extent. Again, the injection of solutions of certain proteids obtained from the thymus, testis, and other organs, will in rabbits cause almost universal thrombosis, and in dogs the clotting is confined almost exclusively to the portal system (Wooldridge[1]).

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[1] This occurs first in the centre of the clot. Cases have been described in which a new central channel has been found through the centre of the clot. The precise nature of the manner in which the fibrin is dissolved is unknown.

[2] Erichsen's *Surgery*, ninth edit. edited by Marcus Beck, vol. i. p. 184. It is, however, possible in the case of purulent discharge that the fever may be caused by the absorption of albumoses formed by the disintegration of pus cells (*see* Pus).

[3] Injection of fibrin-ferment into the circulation has been shown to cause death from intravascular clotting, especially of the pulmonary system, by Edelberg, Köhler, and Birk. Birk's paper (*Inaug. Dissert.* Dorpat, 1880) will be found summarised in *Maly's Jahresbericht*, vol. xi. 1881, where also the references to other works on this subject will be found.

In still another class of cases, intravascular coagulation occurs from the introduction of foreign particles into the circulation. These may be introduced for experimental purposes from without, or the plugs or emboli from which the clotting starts may be detached portions of clots from other parts of the circulation, detached fragments of vegetations from diseased valves of the heart, as after rheumatic fever, &c.

Lastly, a pathological condition of the vascular lining may act in the same way as a foreign body, and cause clotting in the vascular contents; this change may be of the nature of inflammation (arteritis, phlebitis, &c.), or of degeneration (atheroma), or a break in the continuity of the endothelial lining, such as occurs within an aneurism.

There are other pathological conditions in which the blood clots less readily than normally; for instance, in diseases of a pyæmic or acute specific nature (measles, small-pox, scarlet fever), the blood is dark coloured and clots with difficulty, when removed from the body or after death, and the yield of fibrin is small.

Blood rich in fibrin-yielding elements often clots slowly. This is seen in rheumatism, pneumonia, and other acute inflammatory disorders. In all these cases the clot has the buffy coat previously described.

In hæmophilia, a congenital disease characterised by a tendency to immoderate bleedings, whether spontaneous or traumatic, it has been supposed that the affection is due to the lack of fibrin-yielding constituents in the blood. The blood, however, clots when shed and is apparently normal. More probably the fault lies in the blood vessels.

In scurvy and in purpura hæmorrhagica no diminution in the fibrin-yielding elements of the blood has been found (Becquerel and Rodier, Busk); there is a certain amount of anæmia, but in both these diseases, as in hæmophilia, there is but little doubt that the tendency to hæmorrhage is the result of morbid changes, not in the blood, but in the blood vessels.

THE BLOOD IN INFLAMMATION

The blood removed from patients suffering from acute inflammatory diseases (pneumonia, pleurisy, rheumatic fever, erysipelas, &c.) clots more slowly than normal, and hence the coagulum shows a buffy coat. The amount of fibrin, which is normally in man 2·5 per 1,000, may increase to as much as 10 per 1,000 in marked cases. This is accompanied by an increase in the amount of serum-globulin in the serum. In other words, both the globulins (fibrinogen and serum-globulin) are in excess.

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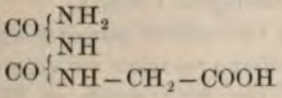
[1] Wooldridge, *Croonian Lecture, Royal Soc.* 1886.

There is also leucocytosis or increase of the white corpuscles, and so there is an increase in the cell-globulin or fibrin-ferment, which in part, no doubt, accounts for the increased amount of globulin in the serum.

The increase of leucocytes is a point which has been very fully worked out by T. P. Gostling.[1] It was originally observed by Pierry in 1837, and later by Virchow, Nasse, and Malassez. Gostling found that the white corpuscles were especially increased in suppurative inflammations, more particularly when accompanied by tension; and that when the tension was relieved, as by opening the abscess, the leucocytosis diminished. He therefore suggests that absorption of leucocytes from the inflamed area in the neighbourhood of the abscess will in part explain the phenomena observed.

It will be seen in the foregoing paragraphs that *rheumatism* is included with other forms of inflammatory disorder. There is no doubt, however, that rheumatism is a specific disease, in which inflammation is more prone to attack the joints than any other part, and in which a very rapid destruction of the red corpuscles occurs; in all probability, the disease is produced by a special poison of a chemical (i.e. non-bacterial) nature. The hypothesis has been advanced that lactic acid in the blood is the *materies morbi*, but this has never been satisfactorily demonstrated. Although we have no positive knowledge of the poison, we at any rate possess the negative information that it is not uric acid, and so rheumatism and rheumatoid arthritis are easily distinguishable from *gout*, where undoubtedly sodium urate is the poison (Garrod). The percentage of uric acid in the serum has in gout been found to vary from 0·004 to 0·175. Oxalic acid has also been found (Garrod). (For the method of demonstrating the existence of excess of uric acid in gouty blood, *see* p. 252.)

Latham's theory of the formation of uric acid in gout[2] is as follows: The glycocine, instead of undergoing its normal change into urea, combines in the liver with two molecules of urea (derived from leucine and other amido-acids), and forms the compound



$$\begin{array}{l}\mathrm{CO}\left\{\begin{array}{l}\mathrm{NH_2}\\ \mathrm{NH}\end{array}\right.\\ \mathrm{CO}\left\{\mathrm{NH-CH_2-COOH}\right.\end{array}$$

which is allied to biuret and allophanic ether.

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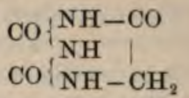
[1] *Medico-Chirurgical Trans.* vol. lxix. (1886), p. 183.

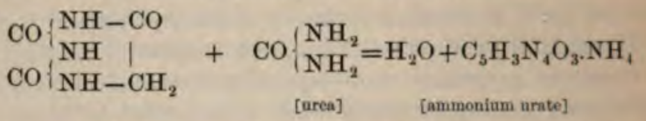
[2] *Lancet*, 1884, vol. i. p. 485; 1885, vol. i. p. 1120.

This substance is dehydrated and forms



$$\begin{array}{l} \mathrm{CO}\left\{\begin{array}{l}\mathrm{NH-CO}\\ \mathrm{NH}\quad |\end{array}\right. \\ \mathrm{CO}\left\{\mathrm{NH-CH_2}\right. \end{array}$$

which, like hydantoin, is soluble in the blood, passes to the kidneys, and there unites with another molecule of urea to form ammonium urate,



$$\begin{array}{l} \mathrm{CO}\left\{\begin{array}{l}\mathrm{NH-CO}\\ \mathrm{NH}\quad |\end{array}\right. \\ \mathrm{CO}\left\{\mathrm{NH-CH_2}\right. \end{array} + \underset{\text{[urea]}}{\mathrm{CO}\left\{\begin{array}{l}\mathrm{NH_2}\\ \mathrm{NH_2}\end{array}\right.} = \mathrm{H_2O} + \underset{\text{[ammonium urate]}}{\mathrm{C_5H_3N_4O_3.NH_4}}$$

which is excreted ; but a portion passing into the general circulation is converted in the blood into sodium urate.

PARASITES IN THE BLOOD

The *Filaria sanguinis hominis* is a nematoid worm found in the blood and urine of patients suffering from chyluria ; the name of the disease is given to it on account of the chyle-like condition of the urine. The disease is also accompanied with swellings under the skin, especially of the scrotum, containing chylous fluid and sometimes discharging the same. The disease affects inhabitants of India and China.

So far as has been ascertained, the presence of the hæmatozoon is the only abnormal feature of the blood ; nothing is known of any changes in the composition of the blood which it may cause.

The *Bilharzia hæmatobia* belongs to the trematode (fluke) family of worms. It is found in the blood of patients suffering from a disease known as Egyptian chlorosis,[1] an endemic hæmaturia occurring not only in Egypt, but at the Cape, the Mauritius, and in South America. The parasite inhabits chiefly the branches of the portal system, and the small veins of the pelvis of the ureter, the ureter, and bladder. The disease is accompanied with hæmaturia (blood in the urine), and it is this constant drain that causes the anæmic condition.

The condition of the urine in these two diseases will be described in a later chapter (*see* Pathological Urines).

These are the only two animal parasites which are known to occur in the blood ; the remaining parasites are vegetable in nature.

Zymotic diseases.—The germ theory of disease teaches that the zymotic diseases, or acute specific diseases, as they are sometimes called, are produced by certain low forms of vegetable life called bacteria, bacilli, micrococci, &c., and their contagiousness consists in the transference of these bacteria or their spores from one person to another. The constant coexistence of a bacterial growth with some of these diseases has been proved, but in many other cases the existence of such germs is merely a matter of inference from the resemblance of the disease to other diseases, in which the existence of a specific bacterium has been proved. The various bacterial growths are distinguished from one another by the shape and size of the bacteria themselves, by the way in which they grow upon certain nutritive media (gelatine, agar-agar, blood-serum, and the like), and by the fact that when a pure cultivation is introduced into another animal a certain set of symptoms is invariably produced.

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[1] Egyptian chlorosis is sometimes caused not by the Bilharzia, but by a nematoid worm, the Dochmius duodenalis. This animal inhabits the duodenum, and the anæmia is caused by leech-like bleedings by means of thousands of these creatures.

In certain acute specific diseases the seat of the bacterial growth is the intestine (cholera,[1] typhoid fever); in others the throat (diphtheria, &c.); in others the skin, or subcutaneous tissues (erysipelas, &c.); in others again, other organs may be the points specially attacked. There are two diseases in which the presence of such germs in the blood has been clearly demonstrated, viz. the spirillum of relapsing fever, and the bacillus anthracis of splenic fever and malignant pustule (woolsorter's disease).

Malaria.—The discovery of the bacillus malariæ placed ague and the various forms of malarial (intermittent) fever among the acute specific diseases. This was a bacillus found in the blood of malarial patients by Klebs and Crudeli, but upon further investigation it was found that it did not fulfil the different conditions which prove the dependence of a disease upon a micro-organism. These conditions, as laid down by Koch, are as follows:—

1. The micro-organism must always be present in the animal suffering from the disease in question.

2. The micro-organism must be cultivated for several successive generations in such a manner as to exclude other micro-organisms and other poisons; and on the introduction of such a pure cultivation into the body of a healthy animal susceptible to the disease, it produces the disease in that animal.

3. It is necessary that the second animal so affected should show in its body the same micro-organisms.

The bacillus malariæ and several other micro-organisms discovered in malarial blood from time to time have not been found to fulfil these conditions. Marchiafava and Celli[1] found that the red blood discs of patients affected with malaria contain peculiar homogeneous bodies possessed of amœboid movements. They call them *hæmo-plasmodium malariæ*. Sometimes these plasmodia include pigment granules assimilated from the pigment of the blood discs. Blood containing the plasmodia is capable of producing intermittent fever in man after intravenous injection, and the blood corpuscles of the person so infected again contain the plasmodia. It is not, however, by any means certain that the hæmo-plasmodium is the cause of the disease. The pigment produced apparently from the red discs by these peculiar bodies is black in colour, and accumulates in and around the smaller vessels of the brain, liver, spleen, and marrow of the bones.[2] There is considerable doubt as to the precise nature of this pigment, and its presence in the blood is called melanæmia. In the disease known as acute tuberculosis, melanæmia also sometimes occurs.

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[1] Owing to the abundant transudations from the alimentary canal in cholera, the blood becomes relatively rich in solid constituents, and may in severe cases even have a viscid consistency.

Phthisis.—This is associated with the presence in the lungs of the tubercle bacillus. The very remarkable statement has been recently made by E. Freund,[3] that the tissues, pus, and blood of tuberculous patients contain cellulose.

Septicæmia and Pyæmia.—The various forms of blood poisoning known by these names are undoubtedly caused by poisons generated during putrefaction, and this is a process which is caused by the activity and growth of micro-organisms. Still, no satisfactory evidence of the constant presence of bacteria in the blood has ever been advanced in man. Klein[4] has occasionally found minute bacilli in the blood vessels of the swollen lymphatic glands, and Koch has described a somewhat smaller bacillus in the blood of mice suffering from a special form of septicæmia, and various kinds of micrococci in certain pyæmic processes in mice and rabbits.

The question here arises, how do bacteria produce their effects? In a few cases, as in those just quoted from Klein, they seem to act mechanically by blocking the vessels and so hindering the circulation through the infected part; but in by far the greater number of cases of blood poisoning and of acute specific disease they produce a chemical poison, in the same way as yeast produces alcohol and carbonic acid from a solution of sugar, and it is the presence of this poison in the blood stream that causes the widespread general effects throughout the body. One of the earliest successful efforts to obtain such a poison was made by Panum and Schmidt (*see* p. 172). A substance called sepsin (now known to be impure) was separated from putrefying blood, and when injected into the blood stream produced the characteristic symptoms of septicæmia. Since then it has been discovered that during putrefaction processes, substances of the nature of alkaloids, called ptomaines and leucomaines, are formed. There is no doubt that in many cases it is poisons of this nature produced by bacteria that cause the diseases of which we have been speaking (*see* more fully Chapters XII and XIII). It must also be remembered that in other cases the poison may be proteid in nature (*see* Chapter X, Proteids as Poisons, p. 137). For some points respecting hæmoglobin crystals in septic diseases *see* p. 315.

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[1] *Fortschritte d. Med.* Nos. xi. xviii. and xxiv. 1885.

[2] Arnstein, *Virchow's Arch.* lxi. 494.

[3] E. Freund, *Wiener med. Jahrb.* 1886, p. 335.

[4] *Micro-organisms and Disease*, p. 120.

THE BLOOD IN DISEASES OF VARIOUS ORGANS

The changes which occur in the blood secondarily to diseases of other organs have been in part already alluded to. In chronic diseases there is invariably anæmia, and in inflammatory diseases the characteristic changes already described. In some diseases of the *heart* and also of the *lungs* there is imperfect aëration of the blood leading to blueness (cyanosis) of the parts most distant from the central circulatory organs. Local patches of cyanosis may occur before the onset of gangrene, and also in certain vasomotor affections (Raynaud's disease).

In affections of the liver, in which there is obstruction in the bile ducts so that that secretion cannot get into the intestine, bile pigment and bile salts enter the circulation, stain the skin and mucous membranes yellow or, in marked cases, brown, and pass into the urine. In some cases *jaundice* occurs when there is no stoppage in the bile ducts; this is called non-obstructive jaundice, or sometimes blood jaundice. In these cases the bile pigments and not the bile salts occur in the circulation. Boerhaave and Morgagni long ago suggested that the jaundice in these latter cases was the result of *suspended secretion*, and the consequent accumulation of the elements of the bile in the blood. Bile acids, however, are never found in normal blood, and although many have searched for bile pigments, no satisfactory evidence of their constant presence in the blood has ever been adduced; the liver, therefore, is not concerned merely in excreting the elements of the bile from the blood, but it actually forms the acids and the pigment within its own cells. Extirpation of the liver, moreover, never leads to the accumulation of the constituents of the bile in the blood. The explanation originally given by Frerichs, and now very generally accepted, of the way in which non-obstructive jaundice is produced is as follows:

Under normal circumstances a very little of the bile poured out into the intestine leaves the body with the fæces; by far the greater amount is resolved into simpler constituents which are absorbed and carried back to the liver to form a fresh supply of bile; but in certain morbid states the absorbed bile does not undergo the normal metamorphoses, but circulates in the blood staining the tissues. The morbid states that conduce to this result are:—

1. Certain poisons: e.g. those of yellow fever, relapsing fever, pyæmia, &c., snake poison, chloroform.

2. Nervous influences: e.g. sudden fright, concussion of the brain, &c.

3. A deficient supply of oxygen, as in some cases of pneumonia.

4. An excessive secretion of bile, especially when conjoined with constipation.[1]

More recent investigations into the pigments of the urine have shown that all dark brown urines are not necessarily coloured by bilirubin, even although staining of the tissues may be present also. An excess of urobilin in the urine produces a colour very like that of jaundiced urine, but it does not give Gmelin's colour test for the bile pigments. We have already seen that hæmatoidin is produced in extravasations of blood, and that hæmatoidin and bilirubin are identical; moreover, after blood has been extravasated into the tissues in large quantity, the urobilin of the urine is much increased. Urobilin can be artificially obtained from hæmoglobin, hæmatin, and bilirubin by the action of reducing agents (MacMunn [2]). Pathological urobilin differs somewhat from normal urobilin, but it is a product derived from hæmatin and not from the bile pigments.[3] We have here, then, instances of brown pigments staining the tissues and urine produced in the blood and not derived directly from the bile, and these cases at first sight look like jaundice. It is possible, that on further investigation it may be found that certain of the cases described as non-obstructive jaundice are not due to a liver affection at all, but that the brown pigment is produced in the stagnant blood of extravasations, or under the influence of certain poisons in the blood stream itself. As illustrating this latter possibility, it may be adduced that injection of the blood of one animal into the vessels of an animal of a different species often produces a breaking up of the blood corpuscles, and the appearance of dark brown pigment staining the tissues and passing into the urine; the so-called jaundice of newly born infants also is often produced by the forcible expression of the placenta immediately after delivery; this sends an abnormal amount of blood into the circulation, and thereby may possibly cause a break-down of some of its corpuscular elements. The pathology of this disease is, however, at present very obscure. Neumann[1] regards it as a true hæmatogenous jaundice, while Halberstamm[2] regards it as hepatogenous, finding not only bile pigment but bile acids also in the urine and pericardial fluids.

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[1] The foregoing account of non-obstructive jaundice is taken from Dr. Murchison's work on the *Diseases of the Liver*.

[2] *See* McKendrick's *Physiology*, p. 131.

[3] MacMunn, *Proc. Physiol. Soc.* 1888, p. vi.

Death from cholæmia.—Patients suffering from jaundice are often attacked with delirium, coma, convulsions, and indications of profound prostration (the typhoid state); in this condition, death may occur. These cases are generally cases of non-obstructive jaundice, and there is no doubt that some alteration in the blood produces excitation and finally exhaustion of various nerve centres. The symptoms are commonly attributed to poisoning with bile, but Frerichs has repeatedly injected bile into the circulation of dogs without producing ill results, and there is ample proof in cases of obstructive jaundice, that the blood of human beings may be saturated with bile for months, or even years, without cerebral symptoms resulting. Dr. Austin Flint has stated that the poison is cholesterin, one of the constituents of the bile; but the cases and experiments just mentioned bear just as strongly against this view as against the somewhat more vague statement that the bile is the poison. Murchison considers that the cause of death in cholæmia is the same as that in uræmia; the liver performs a large amount of the work which is finished in the kidneys, and whenever the liver or kidneys stop work, urea and its antecedents circulate in undue quantity in the blood, and hence the symptoms of poisoning, and, in severe cases, death. One other possibility suggests itself to one, and that is that the cerebral symptoms are not necessarily due to the jaundice, but that both are the results of a poison circulating in the blood; possibly (in the acute specific diseases and pyæmia) of a nature of an alkaloid or poisonous proteid produced by the vital processes of certain micro-organisms.

Another disease of the liver which demands special mention is *acute yellow atrophy*. This disease results in stoppage of the work of the liver owing to rapid fatty degeneration of that organ. There is non-obstructive jaundice, and death occurs after the onset of delirium, convulsions, and deep coma. Very similar symptoms and atrophy of the liver occur in phosphorus poisoning. A distinction between the two disorders is stated to be the occurrence of large quantities of leucine and tyrosine in the blood and urine in acute yellow atrophy, and the absence of these substances in phosphorus poisoning (*see also* Liver).

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[1] Neumann, *Virchow's Archiv*, cxiv. 3.

[2] *Petersburger med. Wochenschr.* 1886, No. 10.

Diabetes mellitus is a disease in which the glycogenic function of the liver is deranged, and of which the consequence is an accumulation of dextrose in the blood, much of which passes into the urine. Normal blood contains about 0·09 per cent. of dextrose (Pavy[1]), but this may increase to 0·2, 0·3, 0·4, and in severe cases to 0·5 and 0·6 in diabetes.

Diabetic coma is a condition somewhat resembling the condition just described, as occurring in cases of non-obstructive jaundice. Some blood-poison circulating in the brain here also causes the condition, and here also the answer to the question—what is the poison? is somewhat unsatisfactory. It is certainly not the sugar; the peculiar odour of the breath and urine of these patients has led some to suppose that acetone is the poison, and the condition is spoken of as acetonæmia.[2] Petters in fact obtained acetone in the distillate from the urine in such a case.

But again large doses of acetone do not produce the symptoms of diabetic coma; and there is no doubt that many patients have the acetone smell, and are far from being comatose: still the prognosis whenever the smell is present is always very grave.[3]

Acetone does not in all probability exist free in the blood, but is derived from the splitting up of ethyl diacetic acid, or an allied compound.

The formula which would represent its formation in the body is as follows:—

$$\underset{\text{[sodium ethyl diacetate]}}{C_6H_9NaO_3} + 2H_2O = \underset{\text{[acetone]}}{C_3H_6O} + \underset{\text{[alcohol]}}{C_2H_6O} + \underset{\text{[sodium hydrogen carbonate]}}{NaHCO_3}$$

The urine of diabetics gives a red-brown colour with ferric chloride, which disappears on adding hydrochloric acid. This is a reaction of ethyl diacetic acid, and that this substance is probably what is present, is supported by the fact that Le Nobel[4] has found not only acetone but also alcohol in the expired air of diabetics. Certain facts have, however, been adduced which tell against this theory. They will be considered in connection with diabetic urine.

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[1] Pavy, *Croonian Lectures, Roy. Coll. of Physicians*, 1878.

[2] Rupstein, *Centralbl. f. d. med. Wiss.* 1874, No. 55.

[3] A very complete discussion of this question with notes of some hundreds of cases has been published by v. Jaksch (*Ueber Acetonurie*, Berlin, 1885: Hirschwald). The question he has investigated specially is not acetone in the blood but in the urine. He states that diabetics without acetonuria never have diabetic coma. Acetone, however, occurs in the urine in fevers, cancer, starvation, and other conditions; and according to Ephraim (*Inaug. Dissert.* Breslau, 1885: Cohn) even in health in small quantity. *See also* Taniguti (*Zeit. physiol. Chem.* xiv.)

[4] Le Nobel, *Centralbl. f. d. med. Wissensch.* 1884, No. 24.

A lipæmic (fat in the blood) condition undoubtedly occurs in many diabetic patients,[1] but this is not constant, and lipæmia may occur in other conditions than diabetes.[2] In this connection it may also be noted that in diabetes a special form of oxybutyric acid occurs in the urine (*see* Urine), and by some diabetic coma has been attributed to the formation of the lower fatty acids in the blood (Mayer [3]).

Bright's disease.—This is the only disease of the kidney that demands special mention. In addition to an anæmic condition there is a great increase in the amount of urea in the blood. When the elimination of urea is defective or stops altogether, symptoms of poisoning (convulsions, coma, &c.) supervene, and are said to be due to *uræmia* (urea in the blood); but as artificial injection of urea into the circulation produces no uræmic convulsions, we are again met with the difficulty, what is the poison? Frerichs' theory that it is ammonium carbonate is now given up as untenable; and for the present we are obliged to rest content with the vague statement that it is some substance (or substances) constituting an intermediate stage in the formation of urea which produces the symptoms. A ptomaine has been suggested by some; a proteid poison by others, but these are mere suggestions not supported by evidence.

HÆMOGLOBIN CRYSTALS IN SEPTIC DISEASES

If normal blood is drawn from the finger, placed on a slide, and covered, no formation of crystals appears. If, however, a drop of putrid serum is added, crystals of reduced hæmoglobin appear in twenty-four to forty-eight hours.[4] The blood from cases of septicæmia crystallises without the addition of any serum. In cancrum oris, in pyæmia (to a less degree), and in erysipelas (especially if the blood is taken from the red patches) the same is observed. These phenomena are probably due to the presence or formation of some ferment produced either by the growth of bacteria, or in leucocythæmia, when the same crystalline tendency of the blood is present, by the disintegration of animal cells. This ferment produces first a deoxidising action on the oxyhæmoglobin, then its exudation into the serum, and lastly crystallisation (C. J. Bond).[5]

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[1] A number of cases illustrating this will be found in Gamgee's *Physiol. Chemistry*, pp. 170–172. *See* also v. Jaksch, *Zeit. f. klin. Med.* xi. 307.

[2] I have myself notes of marked lipæmia in one case of Bright's disease where there was no diabetes.

[3] *Arch. f. exp. Path. u. Pharmakol.* xxi. 119.

[4] Copeman considers that this is characteristic of human blood, the blood of other animals yielding oxyhæmoglobin crystals (*Brit. Med. Journ.* vol. ii. 1889, p. 190). Plaxton, however, has not succeeded in confirming this statement (*Ibid.* vol. ii. 1890, p. 113).

[5] *Lancet*, vol. ii. 1887, pp. 509, 557.

CHAPTER XVII

THE BLOOD OF INVERTEBRATE ANIMALS

INVERTEBRATE animals present in their vascular systems fluids which differ greatly from one another, and from the blood of vertebrates.

The lowest groups in the animal kingdom, the Protozoa and the Cœlentera, possess no cœlom or body cavity, and therefore no vascular system; they obtain food and oxygen direct from the water they inhabit; in the case of the Cœlentera, the water enters the enteric cavity freely. Many degenerate animals of higher groups, such as the tapeworm, have also no vascular system. Then there are other groups, such as the echinoderms (which possess the well-known water-vascular system), the acephalous molluscs (lamellibranchiata), and higher in the series the Tunicates, in which the circulating fluid is principally the sea water or fresh water in which the animal lives; but it contains dissolved in it a certain small quantity of organic substances, and in it float a number of cells like the white corpuscles of vertebrate blood. Blood of this nature may be called *hydrolymph*.

Lastly there are certain groups of invertebrates in which the blood is a highly organised fluid, containing in solution much organic matter, and in suspension numerous corpuscles. There is, however, in most cases no distinction between blood and lymph, and hence this variety of invertebrate blood is sometimes called *hæmolymph*. Worms, most mollùscs, and arthropods possess this variety of blood.

The hydrolymph of invertebrates discharges only one half of the functions of vertebrate blood, carrying nutriment to the tissues and organs, and removing waste products; the respiratory function of the blood is not represented, the gaseous exchanges probably occurring directly between the animal's tissues and the medium it inhabits. In hæmolymph on the other hand it is found that there is a nutritive and a respiratory function taking place. Hæmoglobin is present in the hæmolymph of many animals of the invertebrate subkingdoms, and in many others it is replaced by other respiratory pigments; thus there is the pink pigment hæmerythrin, the blue pigment hæmocyanin, and the green pigment chlorocruorin. But there is this difference to be noted between the blood of vertebrates and that of invertebrates:

that whereas in the former the respiratory pigment is contained in special corpuscles (the coloured corpuscles), in the latter the pigment is dissolved in the plasma; the only corpuscles present being colourless ones. To this rule there are, however, a few exceptions; in some eight invertebrates corpuscles coloured by hæmoglobin, very like the red discs of mammals, have been found.

In the blood of certain groups of animals various other pigments are found (chlorophyll, tetronerythrin, &c.) which have no respiratory functions.

Many of these various forms of invertebrate blood clot when shed like vertebrate blood does. At one time the clot was considered to be merely a mass of adherent corpuscles, or plasmodium of cells (Geddes[1]); but it has since been shown that, in addition to the cells, there is an intercellular substance akin to fibrin which is separated from the plasma; or at least that in many instances this is the case. It is impossible to lay down general laws concerning fluids, which differ so much as do the various forms of blood met with among the invertebrates.

It was just stated that in most invertebrate animals there is no distinction between blood and lymph. There are, however, certain exceptions to this rule: that is to say, there are cases in which the fluid in the cœlom or body cavity is distinct from that in the vessels. The cœlom may be said to contain a fluid comparable to the lymph of vertebrates, while the vessels contain the blood. The lymph or cœlomic fluid never circulates in definite channels or lymphatic vessels as in vertebrates, though the cœlom in some cases may become subdivided into secondary spaces or sinuses.

In some cases the distinction between the two fluids is perfectly distinct; for instance, anyone may, in an earth worm, determine for himself, that a drop of the cœlomic fluid is colourless, while the blood is red. In other cases there is much dispute as to whether or no the vessels communicate with the cœlom,[2] and hence doubt has arisen whether the blood and the cœlomic fluid are or are not identical. It is, however, possible that even if communication does exist, the two fluids might still be different from one another; for in vertebrates there is a connection between the cœlom (pleural and peritoneal cavities) and the blood vascular system *via* the stomata and lymphatic vessels, and yet the lymph and the blood are distinct fluids. The following brief statement of the facts in some of the principal groups is, however, all that we have space for here:—

In the Chætopods there is no doubt that blood and cœlomic fluid are distinct: the same may be said for Phoronis, Sipunculus, and other gephyrean worms. With regard to the leeches (Hirudines) the vascular system is in undoubted communication with the cœlom; still there is at least one difference between the fluids in the two cavities: certain large corpuscles found in the sinuses of Clepsine and

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[1] Geddes, *Proc. Roy. Soc.* xxx. 252.

[2] A concise statement of the best ascertained facts in regard to this question will be found in a paper by A. E. Shipley, *Cambridge Philosophical Soc. Proceedings*, vi. 218–220. This paper also enters into a somewhat similar anatomical point, viz. whether the body cavity and nephridia in certain groups open the one into the other.

Pontobdella are not found in the blood, probably because they are too large to pass through the communicating channels (Bourne).[1] In the nemertine worms the sinuses appear to differ in origin from those in the leeches, not being cœlomic but archi-cœlomic (i.e. the form representing the remnants of the archicœl or segmentation cavity—Hubrecht[2]), and there appears to be no connection between them and the vascular system. Another group separated a long way from these classes of worms, and in which communications exist between the vascular system and the cœlom, is the Echinodermata. Hamann and Koehler showed this first in Spatangids, and Perrier and other French naturalists have shown that the same is true throughout the Echinodermata.

It will be now convenient to take up the chief invertebrate phyla, one by one, and to describe the characters of the blood as it occurs in each.

THE BLOOD OF ECHINODERMS

This is of the nature of hydrolymph, i.e. a watery fluid holding in solution saline substances (derived from the sea water) and a very small quantity of albuminous material. In it float numerous amœboid corpuscles. The following is a brief description of the varieties of corpuscles found in the perivisceral fluid of sea urchins and holothurians (Geddes[3]).

1. Large amœboid cells containing highly refracting spherules of a rich mahogany-brown colour. On exposure to the air this brown colour becomes dingy; but in the vacuum of a mercurial air-pump it rapidly becomes normal again. There is thus considerable probability that this pigment has a respiratory function.

2. Lemon-yellow amœboid corpuscles are also found in certain sea urchins (*Arbocia*, *Dorocidaris*), but are exceedingly abundant in the perivisceral fluid of the Spatangoidea.

3. In the intestinal vessels of Spatangus amœboid corpuscles, varying much in size and containing variously coloured globules (brown, yellow, purple, green, and blue), are found. The nature of these pigments has not been fully worked out, but they are probably lipochromes.

When a drop of the perivisceral fluid is examined microscopically, the cells at first move freely and exhibit amœboid movements. They soon collect into irregular masses and shoot out long processes which bind the cells together.

But the clot is not a mere plasmodium; there is in addition a fibrin-like material which separates from the plasma. This contracts like fibrin after its formation, but in many of its properties it is more like mucin than fibrin (Schäfer[1]).

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[1] *Quart. J. Microsc. Science*, xxiv. 419.

[2] *Ibid.* xxvi. 417.

[3] Geddes in Gamgee's *Physiol. Chem.* p. 184.

In one echinoderm of the ophiurid class, hæmoglobin has been described as occurring dissolved in the blood plasma (Fottinger[2]), and in another (*Thyonella gemmata*, a holothurian) in nucleated oval biconvex corpuscles (Howell[3]).

MacMunn has made numerous observations on the brown colouring matter mentioned as being observed by Geddes in the corpuscles; MacMunn[4] made a spectroscopic examination of the perivisceral fluid of the echinoderms, *Strongylocentrotus lividus*, *Echinus esculentus*, and *Sphæra*. The pigment was found to vary in tint very much, brown, yellow, and red, and it darkened in the air. He named the pigment echinochrome, and considers that it has a respiratory function, the darkening being due to oxidation. In the fresh state it shows no distinct bands, but after the addition of a caustic alkali, or by solution in various solvents (water, glycerine, alcohol, ether, chloroform, &c.), it shows bands which shift in position somewhat with the solvent used; it may, however, be roughly stated that there is one wide shading covering E and another between *b* and F. The solubilities of the pigment are very remarkable; it reminds one of a lipochrome, but differs from other fatty pigments in being soluble in water. The evidence adduced as to its respiratory function does not seem to me to be absolutely conclusive.

THE BLOOD OF WORMS

The blood of worms is coloured in most cases by hæmoglobin dissolved in the plasma, in a few cases contained in special corpuscles. In other worms hæmoglobin is replaced by chlorocruorin, in others still by hæmerythrin.

The following is a list of the different worms arranged in their several classes in which these different pigments have been described:—

A. HÆMOGLOBIN.

Chætopoda.[5]
Lumbricus.
Eunice.
Cirrhatulus.
Nereis.
Limnodrilus.
Lumbriculus.
Nais.
Chætogaster.

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[1] Schäfer, *Proc. Roy. Soc.* xxxiv. 370.

[2] *See* Lankester, *Zool. Anzeiger*, 1883, p. 416.

[3] W. H. Howell, *Studies from the Biol. Lab. Johns Hopkins Univ. Baltimore*, iii. 284.

[4] MacMunn, *Quart. J. Microscopical Science*, October 1885. Charts of the absorption spectra of echinochrome will be found with this paper.

[5] The observations concerning the presence of hæmoglobin in chætopods are all by Lankester (*Journ. of Anat. and Physiol.* 1868, vol. ii. p. 114; *Pflüger's Archiv*, iv. (1871), p. 315; *Proc. Roy. Soc.* xxi. 71).

	Terebella.	Glycera.	
	Tubifex.	Capitella.	
	Arenicola.	Euchytrachus.	Aphrodite.
Gephyrea.	Phoronis (Lankester).		
	Thallasema neptuni (Lankester).		
	Hamingia (Lankester).		
Nemertina.	Polia (Lankester).		
	Other Nemertines (Hubrecht,[1] 1875).		
Hirudinea.	Nephelis (Lankester).		
	Hirudo (Lankester).		
B. CHLOROCRUORIN.			
Chætopoda.	Siphonostomum (Lankester).		
	Sabella (Lankester).		
	Chloronema (Quatrefages).[2]		
	Spirographis (Krukenberg).[3]		
	Branchiomma (Krukenberg).[3]		
C. HÆMERYTHRIN.			
Gephyrea.	Phascoloma (Schwalbe).[4]		
	Sipunculus (Krukenberg).[5]		
	Phoronis (Krukenberg.)[6]		

The worms in which hæmoglobin is present in special corpuscles are the following:—Glycera, Capitella, Phoronis, Thallasema, and Hamingia. In all other cases it is dissolved in the plasma.

Chlorocruorin.—This also is a pigment dissolved in the plasma; it is of a green colour; its decomposition products, however, indicate that it is a pigment of which hæmatin forms the basis as in hæmoglobin. It exists in two conditions, which have been named, from the analogy to hæmoglobin and oxyhæmoglobin, chlorocruorin and oxychlorocruorin. Not only does this occur under the influence of respiratory changes in the body, but by the action of oxidising and reducing agents the same metamorphoses can be produced artificially. Oxychlorocruorin shows spectroscopically two absorption bands, one between C and D, and the other between D and E. Reduced chlorocruorin shows one band having nearly the same position as the first band just mentioned, but it is not so well defined (Lankester[7]).

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[1] Quoted by Lankester, *Zool. Anzeiger*, 1883, p. 416.

[2] Quatrefages, *see* Gamgee, *Phys. Chem.* p. 131.

[3] *Vergl. Phys. Studien*, 2te R. 1 Abth. p. 87.

[4] Schwalbe, 'Kleinere Mittheilungen zur Histologie wirbell. Thiere,' *Archiv f. mikrosk. Anat.* Bonn, Bd. v. 1869, p. 248.

[5] *Vergl. Phys. Studien*, 1ste Reihe, 3te Abth. p. 82. The name hæmerythrin is Krukenberg's.

[6] Described as hæmoglobin by Lankester.

[7] Lankester, *Journ. of Anat. and Physiol.* vol. ii. p. 114; vol. iii. p. 119. MacMunn has also made some observations on the chlorocruorin of Sabella (*Quart. J. Mic. Science*, Oct. 1885). He also examined the blood of a few Serpulæ, and found that though the blood was red it gave bands somewhat like those of chlorocruorin. He considers the pigment to be one intermediate between chlorocruorin and hæmatin.

Hæmerythrin.—Lankester was the first to notice that the pinkish corpuscles of Sipunculus were not coloured by hæmoglobin. Schwalbe made a similar observation in the case of Phascoloma, and Krukenberg is of opinion that the pigment in the corpuscles of Phoronis is the same as in the two worms just mentioned ; he gave the name hæmerythrin to the oxygenated pigment, and hæmerythrogen to the reduced pigment which has a purplish tint. The change occurring as the result of oxidation and deoxidation shows that probably this pigment is a respiratory pigment. It shows no absorption bands, and does not yield hæmin crystals ; but beyond that we know little or nothing about it.

Passing from the pigments to the other constituents of the blood of worms, we find very little is at present known. The cellular elements sink to the bottom of the vessel in which the blood is received, they perhaps stick to each other a little, but there is no real coagulation.

On heating the plasma a heat-coagulum forms at 64°–66°C., and filtering this off, no proteid is left in solution. This is approximately the coagulation-temperature of hæmoglobin ; the same temperature causes a heat-coagulum in the blood of worms containing chlorocruorin. In those containing hæmerythrin there is in addition a second proteid coagulating at 70°C. (Krukenberg).

HÆMOCYANIN

Before proceeding to describe the blood of molluscs and arthropods, it will be here convenient to give a general description of a respiratory proteid of a blue tint, which occurs in both groups, and to which Fredericq[1] has given the name hæmocyanin.

The blue colour of the blood of certain snails (Helix) was noted by Erman (1817) ; in Astacus as well as Helix by Carus[2] (1824) ; in Loligo, Eledone, Sepia, Cancer pagurus, and Helix pomatia by Harless[3] (1847) ; this observer showed that the blue colouration was the effect of exposure to the atmosphere ; and he showed also the presence of copper and a trace of iron in the blood. Genth[4] (1852) ascertained that the blood of Limulus assumed in a similar way a blue tint on exposure to the air, and that it contained copper, and a small amount of iron. Rabuteau and Papillon[5] (1873) showed that in the crab and

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[1] Fredericq, 'Sur l'organisation et la physiologie du poulpe.' Extrait des *Bulletins de l'Académie Royale de Belgique.* 2^{me} Série. T. xlvi. No. 11, 1878.

[2] Carus, C. G., *Von den äussern Lebensbedingungen der weiss- und kalt-blutigen Thiere*, Leipzig, 1824, pp. 85, 86.

[3] Harless, 'Ueber das blaue Blut einiger wirbellosen Thiere, und dessen Kupfergebalt,' *Müller's Archiv*, 1847, p. 48 *et seq.*

[4] Genth, F., 'Ueber die Aschenbestandtheile des Blutes von Limulus Cyclops, *Annalen der Chemie und Pharmacie*, vol. lxxxi. 1852.

[5] Rabuteau and Papillon, *Comptes rendus*, lxxvii. 135.

Octopus similar colour changes occurred. In 1874 Gorup-Besanez[1] added Acanthias and Unio to the above list of animals containing copper in their blood.

Jolyet and Regnard[2] (1877) were the first to advance the opinion that the blue colour was united to a proteid; this was fully worked out by Fredericq[3] in the following year (1878). Since then our knowledge concerning the distribution of hæmocyanin has been added to by Fredericq himself, by Ray Lankester, and by Krukenberg in numerous papers. A full list of all the animals in which it has now been described follows:—The list will be seen to consist chiefly of water-breathing animals, but a few air-breathing animals (snails, scorpions) possess hæmocyanin also.

Crustacea.	Homarus.	Nephrops.
	Astacus.	Eriphia.
	Cancer.	Squilla.
	Carcinus.	Maja.
	Callinectes.	
Arachnida.	Scorpio.	
	Limulus.	
Gastropods.	Cassidaria.	Helix.
	Fissurella.	Murex.
	Haliotis	Turbo.
Cephalopods.	Octopus.	Eledone.
	Sepia.	Loligo.

The properties of hæmocyanin are as follows:—

a. It gives the ordinary proteid reactions.

b. It is coagulated by heat at 65°–66°C. The process of heat coagulation is however slow.

c. It is a globulin; it is incompletely precipitated in dilute solutions by acetic acid, or a stream of carbonic acid. It is also incompletely precipitated by dialysing the salt out from its solutions, or by saturation with sodium chloride. It is completely precipitated by saturation with magnesium sulphate.

d. It exists in two conditions analogous to those of hæmoglobin, viz. oxyhæmocyanin, and reduced hæmocyanin, the former having a blue colour, the latter being colourless; the blood leaving the branchial or pulmonary apparatus is blue; that in the veins is colourless. Reducing and oxidising agents produce the same changes in hæmocyanin removed from the blood.

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[1] Gorup-Besanez, *Lehrbuch der physiologischen Chemie*, Braunschweig, 1874. The blood does not, however, contain hæmocyanin.

[2] Jolyet et Regnard *Arch. de Physiologie*, iv. 600.

[3] Fredericq, *loc*

e. On spectroscopic examination oxyhæmocyanin shows no bands, but only a cutting off of both ends of the spectrum; on reduction the amount of shading is much diminished.

f. It always contains a small quantity of copper, which seems to take the place of the iron of hæmoglobin.

We can now pass on to the remaining invertebrate groups.

THE BLOOD OF MOLLUSCS

Lamellibranchs.—The blood of the Lamellibranchs comes rather under the heading hydrolymph than hæmolymph. It is in most cases colourless; it contains numerous colourless corpuscles. On being shed it deposits a pale, small, colourless clot. C. Schmidt[1] gives the following data concerning the blood of the Anodon or fresh-water mussel:—

Water . .	99·146 per cent.	Albumin . .	0·565 per cent.
Solids . .	0·854 ,,	Salts . .	0·256 ,,
Fibrin . .	0·033 ,,		

The blood of Solen and Arca contains hæmoglobin. This is present in special corpuscles, and not dissolved in the plasma as it is in most invertebrates (Lankester).

Gastropods.—These animals possess a blood much richer in solid constituents than Lamellibranchs. It is, in fact, a hæmolymph. This is well illustrated by the following numbers as estimated by Harless and v. Bibra[2] in the case of the snail Helix pomatia:—

Water . .	85·487 per cent.	Organic solids .	8·393 per cent.
Solids . .	14·513 ,,	Inorganic solids .	6·12 ,,

Hæmoglobin is found in the blood of only one gastropod (Planorbis). The blood of most gastropods contains hæmocyanin; it was in these animals that the presence of a blue colour and of copper in the blood was first shown, though the true significance of these facts was not recognised till later. The hæmolymph of at least two gastropods, Patella and Chiton, contains no hæmocyanin, but has an orange colour. It shows no absorption bands (Krukenberg). Again, in some few cases (Doris, Tethys, Aplysia,[3] and Pleurobranchus) the blood is colourless, and contains only a trace of soluble organic constituents; hence it must be called hydrolymph rather than hæmolymph (Krukenberg).

Coagulation. The only observations on the spontaneous coagulation of the shed blood (hæmolymph) are those of Krukenberg, who states that a jelly-like coagulum forms, and this rapidly becomes fluid again.

Cephalopods.—Here we have a highly organised hæmolymph. It was in the blood of the octopus that Fredericq made the discovery of hæmocyanin. The blood of many other cephalopods contains the same pigment.

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[1] Schmidt, *Lehmann's Physiol. Chem.* iii. 256.

[2] *Müller's Archiv*, 1847, p. 148.

[3] I have had two opportunities of examining the blood of Aplysia, and I can confirm Krukenberg's statement that it is colourless and poor in organic constituents. In one case after filtering off the corpuscles, there was no proteid left in solution; in another there was merely a trace, which was precipitable by saturation with magnesium sulphate, and which, therefore, was probably of the nature of globulin. *See* also Cuénot, *Comptes rend.* cx. 724.

I take the following table from Fredericq's memoir :—

Percentage of	Eledone (Harless)	Sepia (Bert)	Sepia	Octopus	Octopus (Fredericq)
			(Schlossberger)		
Solid matters	7·23	10·9	18·20	12·6	13·689
Salts	2·63	—	3·205	2·225	3·014
Organic matters	4·6	—	—	10·375	10·675
Proteids	—	3·4	—	—	8·9

With regard to the spontaneous coagulation of these fluids, there is no doubt that a clot rapidly forms when the blood is shed. This contracts somewhat in a few hours, squeezing out a small amount of serum. The only corpuscles in the blood of these animals are colourless amœboid ones; and both Fredericq and Krukenberg lend support to Geddes's theory of a plasmodium, i.e. that the clot consists merely of adherent cells. The phenomena of coagulation as described, however, are so closely similar to those in the blood of Crustaceans and of Limulus that I am inclined to believe that in cephalopod blood we have (as in Crustacea) a ferment action converting a previously soluble fibrinogen into a substance very like fibrin.

THE BLOOD OF CRUSTACEA

The blood of a few crustaceans contains hæmoglobin dissolved in the blood plasma. These are as follows :—

Daphnia (Lankester).
Cheirocephalus (Lankester).
Apus (Regnard and Blanchard).
Lernanthropus (Van Beneden).
Clavella (Van Beneden).
Cypris (Regnard and Blanchard).
Marine parasitic Crustacean (undescribed) (Van Beneden). Here the hæmoglobin is said to be contained in a special system of vessels distinct from the blood vessels.

The following is a brief summary of the chief facts ascertained with regard to the hæmolymph of the decapod crustacea.

The blood can be easily obtained by making cuts in the ventral region in the soft integuments between the abdominal segments, or in the claw. It gushes out very readily, and from a large lobster nearly half a pint can as a rule be obtained.

Colour.—The blood which can be seen flowing in the ventral sinus just beneath the skin in this region appears in the vessel to be colourless. The reddish tinge which is present in some specimens when the blood is drawn is so similar to the hue of surrounding parts, that it cannot be perceived through the transparent parts of the skin. The blood when first shed is either nearly colourless, or of a reddish colour from the presence in it of a red pigment presently to be described. It has also an opalescent or milky appearance from the presence of numerous amœboid corpuscles. The milkiness is more marked in blood coming from the claw, than in that from the tail of the same animal. This is due to the cells being more abundant in blood from the former situation. This appearance is however but momentary, for coagulation begins to occur almost instantaneously. This is especially the case with the lobster and crayfish. In the crab coagulation is not so rapid, nor is the ultimate clot so firm and jelly-like.

The blood after being a few moments in contact with the oxygen of the atmosphere acquires an indigo-blue tinge; but the readiness with which this is seen varies in different specimens. The blue colour is due to the oxygenation of a proteid body which exists in solution in the blood plasma; in the reduced state it is colourless; in the oxidised condition it is blue. The name hæmocyanin was given to it by Fredericq.

The variation in the colour of the blood is owing to the admixture of the tint due to hæmocyanin with a varying amount of a red colouring matter. This red pigment has been noted as occurring in the crab by Jolyet and Regnard,[1] and in the lobster by Fredericq[2]; but nothing further was made out about it by these observers. This red pigment is the same as that which exists largely in the exoskeleton and in the hypoderm. It has been called there tetronerythrin, and is one of a class of pigments known as luteins or lipochromes.[3] It can be dissolved out from the blood by alcohol or ether. In Astacus and the lobster the red colour as a rule predominates; but in Nephrops it is present in very small quantities.

Specific Gravity and Reaction.—The specific gravity of the blood is found to vary between 1025 and 1030. Its reaction is always faintly alkaline.

Constituents.—The blood contains the following classes of bodies:—

(1) *Proteids.*

(2) *Salts.*—These resemble those of the water in which the animals live, being more abundant in sea-water than in fresh-water animals. The ash is also found to contain small quantities of iron and copper, the latter being combined with the proteid hæmocyanin (Fredericq).

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[1] Jolyet and Regnard, 'Recherches physiologiques sur la respiration des animaux aquatiques,' *Archives de physiologie*, iv. 600, Paris, 1877.

[2] Fredericq, 'Note sur le sang de l'Homard,' *Extrait des bulletins de l'académie de Belgique*, 2me série, tome xlvii. No. 4, April, 1879.

[3] Merejkowski (*Comptes rend.* xciii. 1029) has found this pigment in 104 species of animals. He considers it may have a respiratory action like hæmoglobin on account of its distribution in the gills. It, however, is not affected by oxidising or reducing agents. Like other lipochromes it bleaches in sunlight, but this occurs equally well in a vacuum. There is no evidence that it is respiratory.

(3) *Extractives.*—Among these are tetronerythrin in variable amount, and fatty bodies, also in variable quantity. There is a small percentage of urea, a fact which has been noted in the case of the crab by Rabuteau and Papillon,[1] and Jolyet and Regnard,[2] and in the lobster by myself.

The following table exhibits the average percentage proportions of these constituents in the blood of four of these animals.

Constituents	Lobster	Crab	Crayfish[3]	Nephrops
Water	93·49	89·92	95·14	89·06
Solids	6·51	10·08	4·86	10·94
Proteids	3·02	6·10	2·19	4·60
Other organic matters .	0·55	1·28	1·54	3·57
Salts	2·94	2·70	1·13	2·77

The foregoing numbers were the averages obtained from the analyses of the blood of three animals in each case, except that of the nephrops, in which six were thus examined. The proteids were estimated by precipitation with alcohol: the blood was allowed to drop direct into alcohol; the constituents of the cells as well as of the blood plasma are therefore included in the foregoing numbers. It is very difficult to estimate the actual dry weight of the cells, because coagulation occurs so rapidly that it is impossible to obtain them free from the fibrin-like substance that is formed; still, by quick filtering, an approximate result can be arrived at, and the cells obtained nearly free from fibrin; this can necessarily only be done in cases where a large amount of blood is readily obtained. In the crab the percentage weight of dried cells was found to be 0·91; and in the lobster 0·73.

Coagulation.—By receiving the blood immediately when shed into very large quantities of neutral salts like magnesium sulphate or sodium chloride, coagulation can be prevented.[4] It has been shown that the clot is not the so-called plasmodium as described by Geddes, but is due to the formation of a body, almost indistinguishable from the fibrin of vertebrate blood, in which the cells are entangled, and that its formation is due to a ferment action upon a proteid fibrinogenous body which exists in the blood plasma. This ferment is derived from the amœboid corpuscles of the blood. As is the case in vertebrata, the serum, that is, the fluid portion that remains when the clot is removed, differs from the plasma by not containing the proteid fibrin factor. Hæmocyanin passes into the serum.

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[1] Rabuteau and Papillon, 'Observations sur quelques liquides de l'organisme des Poissons, des Crustacés, et des Cephalopodes,' *Compt. rend.* lxxvii. 135.

[2] Jolyet and Regnard, *Arch. de Physiologie*, iv. 600.

[3] Witting (*Journal f. prakt. Chemie*, lxxiii. 128) gives the following numbers for Astacus: Water 90·89 Salts 1·55 Organic bodies 7·56.

[4] Dilution of the salted plasma in which the cells have subsided, together with the addition of fibrin-ferment, always produces coagulation.

Corpuscles.—When a drop of blood fresh from the animal is received on a glass slide, covered, and examined with the microscope, the cells are seen in a clear plasma. The blood cells of crustacea have been described and figured by Hewson,[1] Carus,[2] Wharton Jones,[3] Haeckel,[4] Lebert and Robin,[5] and Geddes.[6] Pale cells with amœboid movements and long processes are in all cases described. There are, however, in addition some which contain granules of a yellowish-red colour (tetronerythrin); but these are not constantly present, and are most easily found in those specimens in which the reddish hue of the blood is most marked.

When the drop of blood is examined as above described, the cells at first move freely, and exhibit amœboid movements. They soon become stationary and collect together. They shoot out exceedingly long processes; these help to bind the cells together. But in addition to this, fibres of fibrin with less well defined outline can be clearly seen.[7]

Salts.—One other point may be taken up a little more fully, and this is one which Fredericq[8] has worked out. He has shown that the amount of the inorganic constituents of the blood varies with that in the habitat of the animal, a fresh-water animal like the crayfish containing less than those living in sea water. This is illustrated by the following table:—

Animal	Soluble salts of blood	Soluble salts of water
Crayfish	0·94 per cent.	(fresh water)
Crab	1·65 "	0·9 per cent.
"	3·001 "	3·40 "
Lobster	3·040 "	3·41 "
Maja	3·37 "	3·9 "

The same was found to be true for molluscs. With regard to the solid tissues, they were found to contain less salt than the blood, e.g. the muscles of the lobster contained 0·127 per cent. of soluble salts.

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[1] Hewson, Works edited by Gulliver, *Syd. Soc.* 1846, p. 233.

[2] Carus, C. G., *Von den äussern Lebensbedingungen der weiss- und kalt-blutigen Thiere*, Leipzig, 1824, pp. 85, 86.

[3] T. Wharton Jones, 'The blood corpuscle considered in its different phases of development in the animal series,' *Phil. Trans.* 1846, pp. 90, 91.

[4] E. Haeckel, 'Ueber die Gewebe des Flusskrebses,' *Müller's Archiv*, 1857, p. 510.

[5] Lebert and Robin, 'Kurze Notiz über allegemeine vergl. Anat. niederer Thiere,' *Müller's Archiv*, 1846, p. 121.

[6] P. Geddes, 'On the Coalescence of Amœboid Cells into Plasmodia, and on the so-called Coagulation of Invertebrate Fluids,' *Proc. Royal Soc.* xxx. 252, 1879–80.

[7] A full account of crustacean blood will be found in a paper by myself, *Journ. of Physiol.* vi. 300.

[8] 'Composition saline du sang des animaux marins,' *Libre jubilaire de la soc. de méd. de Gand*, 1884, p. 9.

THE BLOOD OF ARACHNIDA

With regard to the scorpion, we only know that its blood contains hæmocyanin. It is one of the few air-breathing animals that do (Lankester[1]).

Limulus also contains hæmocyanin in abundance;[2] this is stated by Howells[3] to coagulate at a higher temperature (80° C.), and to form a firmer combination with respiratory oxygen than that occurring in crustacean blood. The process of spontaneous clotting that occurs when the blood is shed can, as in the blood of crustacea, be prevented by cold, or admixture with a very large amount of neutral salts. The fibrin formed during coagulation closely resembles that of mammalian blood. In a microscopic preparation of fresh blood it can be seen to be distinct from the cell processes; I am therefore unable to accept Howells's statement that it is composed solely of corpuscles, and my own experiments tend to prove that there is in addition a formation of fibrin from the plasma.

THE BLOOD OF INSECTS

Diptera.—The only observations made on the blood of this class of insects relate to the presence of hæmoglobin in the blood plasma of two of these, viz. the larva of cheironomus (Lankester),[4] and the common house fly (Musca domestica).[5]

Lepidoptera.—A large number of observations have been made by Poulton[6] on the blood of moths and butterflies. The blood is most readily obtainable from the chrysales and caterpillars, but it is apparently the same throughout all the metamorphoses of the animals. The following is a brief summary of the results obtained:—

(*a*) *Reaction.*—The blood is distinctly acid; this is the only known instance in the animal kingdom of the occurrence of acid blood. The acid is a volatile one, and more recent experiments have led Poulton to conclude that it is formic acid.

(*b*) *Corpuscles.*—These are colourless and amœboid.

(*c*) *Colour.*—This is green in most cases; it varies, however, with the food of the animal. The colour is due to chlorophyll derived from the food. The same pigment occurs in other parts of the body. It has apparently no respiratory value, and indeed no respiratory pigment appears to be present. In those animals which live on brightly coloured leaves the blood is brighter than in those which live on leaves of a dull or yellowish-green colour. In the latter case the yellow constituent of chlorophyll (xanthophyll) is present in excess of the green or chlorophyll proper. Spectroscopic examination shows further the close relation between the chlorophyll in the food and that in the blood.

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[1] Lankester, *Quart. Journ. of Microsc. Science*, xxiv. 151.

[2] The total proteids per cent. in the blood averages 6·12.

[3] Howells, *Studies from the Biol. Lab. Johns Hopkins Univ. Baltimore*, 267.

[4] Lankester, *Journ. of Anat. and Physiol.* ii. 114.

[5] Mentioned by MacMunn, 'Animal Chromatology,' *Proc. Birmingham Philosophical Soc.* iii. 385.

[6] E. B. Poulton, *Proc. Roy. Society*, 1885, p. 270 *et seq.*

(*d*) *Proteids.*—These are present and can be precipitated as a white cloud by the addition of alcohol, or by heat (65°–80°C.). This reagent also dissolves out the xanthophyll. Saturation with magnesium sulphate also gives a fairly heavy precipitate; it would thus appear that globulins form a considerable quantity of the proteids present. The chlorophyll, moreover, does not show the great readiness to decompose that plant chlorophyll does. It has, therefore, been suggested that this greater stability may be due to a union more or less intimate between it and one or other of the proteid constituents of the blood.

(*e*) *Coagulation.*—The blood clots after a variable period of time, but generally darkens in about five minutes, ultimately forming a black solid clot, which is due to oxidation. If fresh blood be sealed in a glass tube, it remains unclotted for a month or more; if a small quantity of air be included with the blood, a thin black film forms on its surface and the action then ceases. On removing this crust a new one forms on the surface exposed to the air. This black substance is the normal clot, for the injured places on larvæ which have healed are always black, notably the horns of sphinx larvæ which have been rubbed off by others of the same species. This coagulation is not prevented by the addition of sodium sulphate.

In those species which have brown or colourless blood, there is also darkening on oxidation. The darkening is not due to the action of light, as it occurs equally well in the dark.

These observations of Poulton's are of exceedingly great interest, as on so many points the blood of these animals stands in striking contrast to what occurs elsewhere in the animal kingdom.

Fredericq[1] has also made a few observations on the same blood. He examined the blood of the larva of Oryctes nasicornis. This is colourless, contains colourless corpuscles, gives a precipitate with sodium chloride or magnesium sulphate, is coagulated by the temperature of 55° C., and spontaneously coagulates when shed and exposed to the air. The browning due to oxidation, already mentioned, was observed by Fredericq, and he, like Poulton, regards this phenomenon as not at all analogous to the colour changes due to oxidation in other animals, of the respiratory pigments which we have already fully considered; as when once formed neither air-pump nor reducing agent will remove the brown colour. It is simply the colour of the clot formed.

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[1] Fredericq, 'Sur le sang des insectes,' *Bull. acad. roy. de Belgique*, iii. sér. 1, No. 4, April 1881. Fredericq's observations were, therefore, made previous to the publication of Poulton's results.

Krukenberg[1] has also noted the blackening of the blood of insects whe shed. He applies the term *melanosis* to the process. He found it to occ only in the blood of the lepidoptera (butterflies and moths), but also in t certain coleoptera (beetles). Though he observed also the green colour blood in certain chrysales, he does not seem to have recognised that chlorophyll.

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[1] **Krukenberg**, 'Ueber die Hydrophilus lymphe, &c.' *Verhandl. d. nat.-medic.* *zu Heidelberg*, N.F. vol. iii. Heft 1; and 'Zur Kentniss der Serumfarbstoffe,' *Sit* *berichte der Jenaischen Gesell. f. Medicin*, 1885.

CHAPTER XVIII

LYMPH AND ALLIED FLUIDS.

INTRODUCTORY

In vertebrate animals there is, in addition to the blood vessels, a system of vessels and spaces which is called the lymph-vascular system, or the lymphatic system. The fluid contained in this system is called lymph. On the course of many of the lymphatic vessels are found masses of lymphoid or adenoid tissue, which are called lymphatic nodules and lymphatic glands. Lymph is derived from the blood plasma; as the blood circulates in the smaller vessels and capillaries, a certain quantity of the plasma, or liquor sanguinis, diffuses through the thin walls of these vessels, and bathes the tissue elements so that they are brought into very close relation with the nutrient fluid. This exuded fluid, or lymph, contains also a few white corpuscles, which have emigrated into the tissues from the blood vessels. The lymph collects in the connective tissue spaces, and from these passes into lymphatic capillaries, which by uniting form larger vessels accompanying the veins, and these all open into the large lymphatic trunk, or thoracic duct, which opens into the venous system at the junction of the subclavian and jugular veins. During this course the vessels may enter one or more lymphatic glands; the lymphatic vessel which leaves the lymphatic gland (efferent lymphatic vessel) contains a greater number of colourless corpuscles (lymph corpuscles) than was present in the afferent lymphatic vessels; the lymphatic nodules and glands are the places where these corpuscles are formed, and by amœboid movements they work their way into the lymph path, and are carried on first into the lymphatic circulation, and ultimately into the blood stream, where they receive the name of white blood corpuscles. The chemistry of these corpuscles we have already considered (p. 257). The liquid in which these corpuscles float may be called the *lymph plasma*. It may be briefly described as diluted blood plasma. The saline constituents of lymph plasma and blood plasma are alike both as regards quality and quantity. The organic, and especially the proteid, constituents are alike in kind in both varieties of plasma but are much less abundant in lymph plasma than in blood plasma The lymph, however, contains a somewhat larger proportion of the products of combustion of the tissues, such as carbonic acid and urea, than the blood does.

During digestion the lymphatic vessels of the intestine take up certain of the products of digestion, among others fat in a finely divided condition. This gives to the lymph in this situation a milky appearance, and the vessels are consequently called *lacteals*, and their contents *chyle*. During the intervals between digestion the lacteals, however, contain ordinary lymph.

The serous cavities are in close relationship with the vascular system from the point of view of embryology. They may be considered as large lymph spaces, and during health they contain a small quantity of fluid which is lymph. The name, serous membrane, is not altogether a good one, as it suggests that the fluid they contain is serum. In the serous membranes are minute holes, or stomata, which open into lymphatic vessels, and thus the interior of serous membranes and of the blood vascular system are brought into communication with one another.

The synovial cavities surrounding the joints, the cerebrospinal cavity in the interior of the brain and spinal cord, and the anterior chamber of the eye, contain somewhat similar fluids, which are called respectively, synovia, cerebrospinal fluid, and aqueous humour.

Under certain pathological conditions the amount of fluid in these different situations is increased, so producing the various forms of dropsy.

The causes of dropsy may be briefly summarised as follows:—

It may be due to:

1. Disordered conditions of the circulation. Obstruction to the flow of blood through the heart, as in certain valvular diseases of that organ[1]; or to the flow of blood through veins or lymphatics, as by the pressure of tumours upon them, will, by hindering the free return of fluid to the heart, produce increased pressure in the capillaries, and so increased exudation of lymph. In the case of obstruction in veins, the dropsy may be localised or more or less general; for instance, a clot in one femoral vein will only cause dropsy of one lower limb, while pressure upon the vena cava inferior would cause dropsy of all the lower part of the body. In heart disease dropsy will always be more or less general, but as a rule manifests itself most in the lower part of the body, where there is most gravity to overcome.

2. Disordered conditions of the blood. The best examples of this are Bright's disease, and certain forms of anæmia where the blood plasma passes more readily than normal through the vascular walls.

3. Disordered conditions of the vessels. This comes into play especially in inflammation. Here not only does the plasma pass more readily than normal through the vessels, but the emigration of white corpuscles is much increased, and

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[1] The cause of dropsy in heart disease is, according to Wooldridge, partly due to an altered condition of the blood (fibrinogen-poisoning), *Proc. Roy. Soc.* xlv. 309, 1889. In this Wooldridge differs from other observers.

red corpuscles in small numbers may pass through the vessel walls also. The formation of an abscess is inflammation advanced to such an intense degree that the escaped white corpuscles are so numerous as to form what is known as pus.

In dropsy caused in these three different ways the fluid effused is different from normal lymph. In pressure dropsy the fluid is more watery than normal lymph; in dropsy due to an increased watery condition of the blood the effused lymph is similarly altered. In inflammatory dropsy not only are the lymph corpuscles very numerous, but the solid constituents of the lymph plasma are more abundant than normal.

In addition to all these forms of dropsy, there are further localised dropsies due to the formation of cavities and cysts in organs. These spaces get filled with fluid; thus we have tumours of the membranes of the brain and spinal cord (meningocœles) which become filled with cerebro-spinal fluid, cystic diseases of the ovary, fallopian tubes, kidneys, &c., hydatid tumours of the liver and other organs, and the amniotic fluid which surrounds the embryo, may be also included under this head.

Such, then, is a summary of the various kinds of fluid we have now to take up in detail: first normal lymph and chyle, and the contents of the serous, synovial, and cerebrospinal cavities in health and disease; next the more distantly related fluids that occur in the interior of certain tumours; and lastly the subject of pus.

LYMPH

Small quantities of lymph for microscopical investigation may be obtained from the dorsal lymph sac of the frog, or from any of the lymphatics of the higher animals. Larger quantities for chemical investigation have been obtained from the thoracic duct of anæsthetised animals, or in the case of large animals like the horse from other large lymphatic trunks, for instance, that accompanying the jugular vein; in man lymph has been obtained from cases of lymphatic fistula; Hensen and Dähnhardt[1] observed the properties of the lymph obtained from such a fistula in the thigh.

Lymph, like plasma, is of a faintly yellow colour, and has an alkaline reaction; its specific gravity varies between 1012 and 1022. On microscopic examination it shows a number of amœboid cells similar to white blood corpuscles; this number varies in different parts of the lymphatic system, as has already been described (p. 331).

When shed, the lymph in from 3–20 minutes coagulates, yielding from 0·04 to 0·08 per cent. of fibrin (blood plasma yields a larger quantity, 0·22–0·4 per cent.); the liquid residue may be called lymph-serum, and like blood-serum it contains proteids (serum-globulin and serum-albumin), extractives, salts, and gases. The last three classes of constituents are also similar to those occurring in blood-serum; the products of the combustion of the tissues, e.g. urea and carbonic acid, are, however, more abundant in lymph than in blood, while the other organic constituents are less abundant in lymph than in blood.

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[1] *Arch. f. pathol. Anat.* vol. xxxvii. pp. 55 and 68.

When commercial peptone is injected into the blood-stream of certain animals (e.g. the dog), the blood when shed does not coagulate; the same is true for the lymph of such animals.[1]

The following table contrasts the composition of blood plasma with that of lymph:—

	1,000 parts of human blood plasma contain:—[2]	1,000 parts of human lymph contain:—[3]
Water	902·90	986·34
Solids	97·10	13·66
(*a*) Proteids—		—
Fibrin . . .	4·05	1·07
Other proteids . .	78·84	2·30
(*b*) Extractives . .	5·66	1·31
(*c*) Inorganic salts .	8·55	8·78

This table illustrates numerically the various points which have been already mentioned, especially the great diminution in the organic constituents of the lymph as compared with plasma, while the inorganic constituents are approximately the same in the two fluids.

Not only are the total inorganic constituents equal in the two fluids, but the same salts are present in approximately equal proportion, sodium chloride in each being the most abundant; this is illustrated numerically in the next table.

1,000 parts of human plasma contain (C. Schmidt):—	
NaCl	5·54
Na_2PO_4	0·27
Na_2O	1·53
KCl	0·36
K_2SO_4	0·28
$Ca_3(PO_4)_2$ . . .	0·30
$Mg_3(PO_4)_2$ . . .	0·22

1,000 parts of human lymph contain (Hensen and Dähnhardt):—		
Soluble	NaCl . .	6·14
	Na_2O . .	0·57
	K_2O . .	0·49
	CO_2 . .	0·63
	SO_3, P_2O_5 & loss	0·22
Insoluble	CaO . .	0·13
	MgO . .	0·01
	Fe_2O_3 . .	0·006
	P_2O_5 . .	0·118
	CO_2 . .	0·015
	$MgCO_3$ & loss .	0·021

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[1] Fano, *Du Bois Reymond's Archiv f. Physiol.* 1881, p. 277.

[2] C. Schmidt and Lehmann.

[3] Hensen and Dähnhardt. The above table gives the averages of three analyses. The total solids are rather low in this case; the average from other cases gives from 30–40 parts per 1,000.

Similar tables might be quoted of analyses of the lymph from other animals; the chief points, however, are all sufficiently shown by the example given.[1]

The next table illustrates the point already mentioned as to the greater quantity of urea in lymph as compared with the blood (Wurtz[2]).

Animal	Percentage of Urea		
	Blood	Lymph	Chyle
Dog	0·009	0·016	—
Cow	0·019	0·019	0·019
Horse	—	0·012	—
Bull	—	0·021	0·019

Observations have been made in dropsical fluids which show that the relation between the amount of serum-albumin and serum-globulin is very constant in the same person or animal, and is the same approximately in the blood-serum and in the effused fluids, though the total amount of proteids is much less in the effusions than in the serum.

If a=percentage of albumin
and b=percentage of globulin,

Then $\frac{a}{b}$ is called the proteid quotient.

The proteid quotient is also equal in the same animal in the serum, lymph, and chyle. Salvioli[3] has shown this to be the case in dogs. This is a somewhat important point; it shows that there is no difference in the rate of diffusion of the two proteids in the living animal, for Gottwalt[4] found that globulin diffuses more slowly than albumin through dead animal membranes. (Compare p. 15)

CHYLE

Chyle is the name given to the fluid contents of the intestinal lymphatics or lacteals during digestion. It may be briefly described as lymph *plus* certain materials (especially fat) which have passed into these vessels from the intestines.

Microscopic examination shows that chyle contains lymph corpuscles and fat globules in a minute state of subdivision.

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[1] These tables will be found in Hoppe-Seyler's *Physiol. Chemie*, pp. 592–3.

[2] Wurtz, *Comptes rendus*, July 1859. I take the table from Gamgee's *Physiol. Chem* p. 224.

[3] Salvioli, *Du Bois Reymond's Archiv f. Physiol.* 1881, p. 269.

[4] *Zeit. physiol. Chem.* iv. 423.

The following table, somewhat abbreviated, is taken from Hoppe-Seyler;[1] the numbers are parts per 1,000.

Constituents	Chyle of Dog[2]	Serum of the same Dog[2]	Chyle of Horse[4]	Human Chyle[5]	Human Chyle[6]
Water	906·77	936·01	956·19	904·8	943 to 958
Solids	96·23	63·99	43·81	95·2	56 to 41
Fibrin	1·11	—	1·27	70·8	11 to 13
Albumin & globulin	21·05	45·24	29·85		
Fat, lecithin, cholesterin.	64·86	6·81	0·53	9·2	25 to 27
Fatty acids & soaps	2·34	2·91	0·28	10·8	—
Other organic substances			2·24		
Mineral salts . . .	7·92	8·76	7·49	4·4	6·25

The most striking fact illustrated by this table is the greater amount of solids in chyle as compared with lymph, and the large percentage of fat. Zawilski found in dogs fed purely on a fatty diet that the chyle might contain as much as 14·6 per cent. of fat. During the active digestion of fat, the blood plasma and serum have a milky appearance produced by the presence in them of excessively minute fat globules.

1,000 parts of the dry residue of the ethereal extract of chyle contained:[7]—

	First Specimen		*Second Specimen*
Cholesterin	113·2		140·9
Lecithin	75·4		88·4
Olein	381·3	811·4	770·7
Palmitin and stearin . . .	430·1		

The chyle also contains a certain proportion of soaps absorbed from the alimentary canal. This statement was originally made by Hoppe-Seyler, and he still maintains[8] the correctness of his earlier observations, which have been questioned by Lebedeff, Röhrig, and Zawilski. In some new experiments he has found in the serum of the horse, ox, and dog, a percentage of fatty acids from soaps varying from 0·05 to 0·12.

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[1] *Physiol. Chemie*, p. 595.

[2] *Ludwig's Arbeiten*, xi. 147.

[3] Analyses by Hoppe-Seyler.

[4] Analysis by Schmidt.

[5] Analyses by Rees, *Phil. Trans.* 1842, p. 81. The chyle was obtained from a decapitated criminal.

[6] Noel Paton. This analysis is not contained in Hoppe-Seyler's table. The chyle was obtained from a patient whose thoracic duct had been ruptured by an operation for tumour in the neck (*Journ. of Physiol.* xi. 109).

[7] Hoppe-Seyler, *Physiol. Chem.* p. 597.

[8] Hoppe-Seyler, *Zeitsch. f physiol. Chem.* viii. 503.

In the chyle[1] he has found 0·225 per cent. of soaps, and 0·723 per cent. of fat.

The increased percentage of proteids in the chyle as compared with the lymph illustrates the fact that the lacteals are not merely concerned in the absorption of fatty, but probably also of albuminous food. In the stomach and intestine the proteids of the food are converted into peptones, substances that diffuse with readiness through living animal membranes; but no peptones[2] (or proteoses, the intermediate products in the formation of peptones) are found in the chyle; during their passage through the intestinal wall, or immediately on entering the lymph or blood stream, they are reconverted into albumin and globulin. Schmidt-Mulheim[3] tied the thoracic duct in dogs, and found that proteids were still absorbed; this, however, does not prove that the lacteals are not normally concerned in the absorption of proteid; it merely shows that animals thus treated can continue to absorb proteid by the other path—the blood vessels.

The intestinal lymphatics are thus concerned in the absorption of fat and of proteids; they, however, apparently take but little part in the absorption of carbohydrate food; the amount of sugar in lymph and chyle is approximately the same as in the blood, and no definite increase occurs when animals are fed on a starchy or saccharine diet (Bernard, v. Mering[4]). Probably, as sugar is so easily diffusible, most of it passes into the more quickly circulating blood stream and is carried off, fresh quantities of blood being then available to carry off more. The blood vessels, moreover, lie immediately beneath the epithelium, and so the sugar never reaches the more centrally situated lacteals of the villi (Heidenhain[5]). By greatly increasing the amount of sugar in the food, however, some does pass into the chyle.[6]

Quantity of Chyle.—From two cases in which the amount of chyle was measured C. Schmidt concluded that for every kilogram of body weight 0·61 kilo of chyle was formed in the 24 hours, of which 0·34 comes from the alimentary canal, and the remaining 0·27 consists of the normal lymph. Hoppe-Seyler[7] is inclined to think that the proportion derived from the intestine is much smaller.

Exudations of Chyle.—Owing to the rupture of the lacteals or of the thoracic duct, or owing to fistulous communications between these parts and other cavities, chyle may pass into the serous cavities, giving rise to chylous dropsy. For Chyluria *see* Urine.

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[1] Obtained from a case of chylous ascites, i.e. escape of chyle into the peritoneal cavity.

[2] This is a statement made by numerous observers; I have confirmed its accuracy in an examination of chyle collected from the thoracic duct of two dogs.

[3] *Du Bois Reymond's Archiv*, 1877, p. 549.

[4] *Ludwig's Arbeiten*, 1877. *Arch. f. Anat. u. Physiol., Physiol. Abth.* 1877, p. 379.

[5] Heidenhain, *Pflüger's Arch.* supplemental volume, 1888, p. 71.

[6] Ginsberg, *Ibid.* xliv. 306.

[7] *Physiol. Chemie*, p. 597.

THE LYMPH IN SEROUS CAVITIES DURING HEALTH

The amount of fluid in these cavities is in health very small; excess finds its way through the stomata into the lymphatic vessels. The fluid is undoubtedly lymph—that is, dilute blood plasma which has exuded from the blood vessels. In dropsical conditions this fluid is much increased, and our knowledge of its properties is almost entirely derived from a study of dropsical fluids. If excess of fluid accumulates in two of these cavities simultaneously, as, for instance, in the peritoneum and the pleura, from alterations in conditions of vascular pressure (e.g. heart disease), it is found that the composition of the two fluids differs to a certain extent. On this ground we hold that the normal lymph which moistens the various serous cavities probably differs in those different cavities in the same way. The differences are quantitative only, not qualitative. After death the pericardium, especially in some animals (e.g. the horse), often contains a considerable quantity of liquid. This accumulation is accounted for by the changes in the circulation immediately preceding death. The liquor pericardii is a liquid which is interesting historically, as so many experiments have been made with it in dealing with the investigation of the causes of the coagulation of the blood (*see* p. 243). The cerebro-spinal cavity is not a serous cavity, and the fluid in it differs markedly from the lymph of the serous cavities. It will be dealt with separately.

DROPSICAL FLUIDS

The modes of causation of dropsy have been already considered (p. 332). These fluids may all be tersely described as lymph in excess and more watery than usual, except in inflammatory dropsy, where the cells and the solid constituents generally are increased.

Nomenclature.—There are certain names given to the various forms of dropsy occurring in different situations:—

Œdema is the name given to the excessive exudation of fluid into the subcutaneous tissues.

Ascites is the name given to a dropsy of the peritoneal cavity.

Hydrocele is the name given to a dropsy of the tunica vaginalis, the serous membrane originally part of the peritoneum that surrounds the testicle.

Hydrothorax is the name given to a dropsy of the pleura.

Hydropericardium to that of the pericardium.

The names of diseases that are inflammatory in nature terminate in the affix *-itis*. Thus there is pericarditis, peritonitis, pleuritis (or pleurisy), &c., and in certain stages of all these diseases there is effusion of fluid.

Reaction.—This is in all cases alkaline.

Colour.—This varies directly with the colour of the blood plasma of the patient, and with the concentration of the effused liquid, but there is always a certain amount of the yellowish-green lipochrome (serum-lutein, *see* p. 253), which can be extracted by means of alcohol.

Specific gravity.—This increases *pari passu* with the amount of solid constituents. Reuss[1] has examined a large number of these effusions; he calls the fluids of inflammatory dropsy, exudations, while the dropsical fluids (i.e. diluted lymph) he terms transudations, and the following are the general conclusions he draws with regard to specific gravity.

Fluids from cases of	peritonitis	1018 or higher	exudations
„ „	pleuritis	1018 „	
„ „	inflammation of skin[2]	1018 „	
„ „	hydrothorax	1015 or lower	transudations
„ „	ascites	. 1012 „	
„ „	œdema	. 1010 „	

Coagulation.—Non-inflammatory dropsical fluids do not coagulate spontaneously, or only with exceeding slowness. When mixed with serum, or contaminated with blood, as they are apt to be in the process of tapping, they, however, do coagulate, forming fibrin.

The reason why they do not clot is that they contain either very few cellular elements or these may be practically absent. The cause of the coagulation of the blood, we have already seen, is the formation of the fibrin-ferment from white corpuscles and blood tablets. When, therefore, blood or serum or a solution of pure fibrin-ferment or cell-globulin, or of some other active globulin like myosinogen (*see* Muscle), is added to one of these dropsical fluids, the fibrinogen contained therein is converted into fibrin (*see* Coagulation of the Blood, p. 241).

The inflammatory fluids or exudations are, however, different; they contain abundance of white corpuscles and invariably clot when shed.

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[1] A Reuss, *Deutsches Arch. f. klin. Med.* xxviii. 317. Hoffmann has made similar observations, *Virchow's Archiv*, lxxiii. 250.

[2] Such as is obtained by blistering.

Sometimes they clot within the serous cavity, the fibrin[1] sticking to the sides of the membrane.

Constituents.—These are the same in kind as those in blood plasma.

a. *Proteids.*—These are fibrinogen, serum-globulin, and serum-albumin.[2]

b. *Extractives.*—This term is used in the sense explained on p. 251. In some cases cholesterin is found in marked excess. Sugar seems to be a fairly constant constituent.

c. *Salts.*—These are alike not only in kind but in actual amount to those in the blood.

The different dropsical fluids differ from one another in their richness in organic constituents, especially in proteids; the pleural fluid is richest in these substances, then the peritoneal, and lastly the fluid of subcutaneous œdema. These facts may be illustrated by the following tables:—

Composition of various dropsical fluids removed after death from a case of albuminuria (C. Schmidt)[3]:—

In parts per 1000	Pleural Fluid	Peritoneal Fluid	Œdema Fluid
Water	963·95	978·91	988·70
Solids	36·05	21·09	11·30
Organic	28·50	11·32	3·60
Inorganic	7·55	9·77	7·70

Composition of various dropsical fluids removed simultaneously from a case of albuminuria (Hoppe-Seyler)[4]:—

In parts per 1000	Pleural Fluid	Peritoneal Fluid	Œdema Fluid
Water	957·59	967·68	982·17
Solids	42·41	32·32	17·83
Proteids	27·82	16·11	3·64
Extractives and Salts . .	14·59	16·21	14·19

These examples illustrate sufficiently well the fact that it is the amount of proteids that varies in these different fluids, the other constituents being fairly constant.

Runeberg[5] examined 77 cases of effusions of different kinds; the amount of total proteids varied from 0·06 to 2·68 per cent.; while the amount of chlorides, the most abundant salts, averaged 1·08, varying only 0·1 per cent. throughout the long series.

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[1] One often hears these strands of fibrin called lymph in the post-mortem room.

[2] The serum-albumin of these fluids like that of serum can by fractional heat-coagulation be differentiated into three proteids (*see* p. 247).

[3] Quoted by Hoppe-Seyler, *Physiol. Chem.* p. 602.

[4] *Physiol. Chemie*, p. 602. Also in *Arch. f. path. Anat.* ix. 257.

[5] Runeberg, *Deutsch. Archiv f. klin. Med.* xxxv. 266.

Runeberg[1] in three cases (I., II., III.), and myself in one case (IV.) of heart disease examined dropsical fluids removed simultaneously from different parts. The following numbers give the percentage of proteids in the different fluids :—

Case I	Case II	Case III	Case IV
Fluid from pleura 0·11	Fluid from peritoneum . . . 2·3	1·64	Fluid from pleura . . 1·48
Fluid from peritoneum . . . 0·12	Œdema fluid . . 0·24	0·20	Œdema fluid 0·33
Fluid from pericardium . . . 0·52			

F. Hoffmann[2] examined a series of thirty cases of ascitic fluid as to the relation between the amount of serum-globulin and serum-albumin. The amount of fibrinogen in these fluids is so small, that practically it may be neglected. He found that the fraction $\frac{\text{serum-albumin}}{\text{serum-globulin}}$, which is called the proteid quotient, is very variable. In eleven of the thirty cases he was able to estimate the proteid quotient in the blood-serum of the same patients ; here, also, he found great variations, and the quotient is generally, both in blood and in effusion, lower than normal.[3] The most interesting point, however, is that the proteid quotient is practically the same in the two fluids, blood and effusion, for the same individual. We have seen previously (p. 335) that the proteid-quotient of normal lymph and chyle is equal to that of the blood. This illustrates to us very forcibly the differences between diffusion through a living membrane and through a dead membrane. Senator, A. Schmidt, and Gottwald, using dead membranes, found that serum-globulin diffuses with greater difficulty than serum-albumin ; but here we see that during life they diffuse with equal rapidity.

These results have been fully corroborated by Pigeaud,[4] and I quote (*see* next page) the numbers obtained by him in two cases of nephritis.

In many cases during the progress of a dropsy it is found necessary to tap the cavity several times ; it is then found that successive

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[1] Runeberg, *Ibid.* xxxiv. 1.

[2] F. Hoffmann, *Arch. f. exper. Path. und Pharm.* xvi. 133.

[3] It appears doubtful whether we can at present say what the normal proteid-quotient is. It is more probable that it exhibits very considerable variations in health.

[4] J. J. Pigeaud, *Over eiwitstoffen in serense vloeistoffen. Doctor. Dissert.* Leiden, 1886; *see* also *Maly's Jahresbericht f. Thierchemie*, xvi. 474.

Case I

Fluid	Total Proteid per cent.	Proteid Quotient $\frac{\text{Albumin}}{\text{Globulin}}$
Blood-serum	5·261	0·664
Pleural fluid	0·808	0·680
Ascitic fluid	0·452	0·686
Œdema fluid	0·212	0·677

Case II

Fluid	Total Proteid per cent.	Proteid Quotient
Blood-serum	5·781	1·056
Pleural fluid	0·900	1·142
Ascitic fluid	0·832	1·122
Œdema fluid	0·775	1·152

dropsical transudations into the same sac present great constancy of composition. Sometimes differences do occur; these differences, and also the differences in the proteid contents of the lymph in the various serous sacs, are, no doubt, dependent on alterations and differences in the mechanical conditions of pressure.

In successive tappings, however, a difference may arise in another way, viz. a certain amount of inflammation may be set up in the serous membrane itself; and this may be the result of irritation produced by a sudden removal of the fluid, or, more frequently, it is the result of using imperfectly cleansed instruments for the operation.

These different points will be illustrated by further analytical data to be given under the various fluids which we now proceed to take up *seriatim*.

PERITONEAL FLUID

This partakes of the general character of dropsical fluids which have just been described.

It is an alkaline fluid of a yellowish tint, and is occasionally, even when quite fresh, somewhat opalescent.[1] It contains few or no corpuscles, and when removed does not coagulate spontaneously, or only very slowly, the process sometimes lasting several days.

Where peritonitis is present, however, colourless corpuscles and epithelium cells of the peritoneum, and, in cases of cancer, cancer-cells[2] also are to be seen in abundance; the fluid coagulates spontaneously and is much richer in proteid-contents than the fluid of simple dropsy.

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[1] This opalescence is not removed by filtration, and microscopically no particles are to be seen to account for it.

[2] H. Quincke, *Deutsch. Arch. f. klin. Med.* xxx. 5.

Some analyses have already been given of the composition of this fluid; the following may now be added, as they illustrate more fully certain other points, which may be conveniently stated in the form of propositions, each of which is followed by illustrative analyses.

1. The fluid removed from the peritoneal sac by successive tappings remains fairly constant in composition.[1]

In parts per 1000	*Case I*		*Case II*[2]		
	1st Tapping	2nd Tapping	Cirrhosis of Liver		
			1st Tapping	2nd Tapping	Removed after Death
Water	952·99	960·49	984·50	982·53	983·33
Solids	47·01	39·51	15·50	17·47	16·67
Proteids	34·90	29·73	6·17	7·73	6·11
Extractives	4·28	3·75	1·25	1·84	3·25
Salts	7·22	5·94	8·46	8·13	8·24

2. The amount of proteids is very variable; the proteid quotient is also variable and apparently does not vary with the cause of the dropsy. This may be illustrated by the following analyses selected from a larger number made by myself.

Case	Reaction	Specific Gravity	Total Proteid per cent.	Serum-Globulin[3]	Serum-Albumin
1. Cirrhosis of liver	In all cases Alkaline	1010	0·955	0·413	0·542
2. Syphilitic disease of liver		1012	0·744	0·252	0·492
3. Cirrhosis of liver		1012	2·021	1·114	0·907
4. Non-inflammatory		1016	2·235	1·516	0·719
5. Heart disease		1016	4·11	1·48	2·63
6. Heart disease[4]		1018	4·334	2·937	1·397

3. In the same case, however, it is found in successive tappings that the total proteid and the proteid quotient remain very constant.

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[1] Scherer, quoted from Hoppe-Seyler's *Physiol. Chem.* p. 602.

[2] Hoppe-Seyler, *Ibid.* p. 603. Other analyses illustrating this point will be also found here.

[3] In these cases the amount of fibrinogen is not given, as it was very small, and it is weighed with the serum-globulin. I am indebted to Dr. Sydney Ringer and his house physicians at Univ. Coll. Hosp. for all these various fluids and many others to be described later on in this chapter. *See* also *Brit. Med. Journ.* vol. ii. 1890, p. 192.

[4] The amount of proteids in transudation fluids from heart disease is generally greater than in other forms of pressure dropsy. This lends some support to Wooldridge's theory, that the blood is partly at fault in such cases (*see* footnote, p. 332).

Case of Bright's Disease complicated with Cirrhosis of the Liver

	Specific Gravity	Total Proteid per cent.	Serum-Globulin	Serum-Albumin
First tapping . . .	1014	2·037	0·7807	1·2563
Second ,, . . .	1015	2·499	0·8960	1·6074
Third ,, . . .	1015	2·401	0·572	1·829
Fourth ,, . . .	1015	2·152	0·703[1]	1·375

4. Even in those cases where, owing to alterations of pressure, the amount of proteid changes, yet the proteid quotient (albumin : globulin) remains practically unaltered.

Case of Cirrhosis of the Liver in a Boy thirteen years old[2]

Date of Tapping	Total Proteid per cent.	Proteid Quotient
July 20, 1885	3·285	1·483
August 25, 1885	0·632	1·573
September 30, 1885	2·368	1·532
November 15, 1885	3·216	1·525
December 27, 1885	2·688	1·486

5. The total proteids in the fluid in cases of peritonitis is increased as compared with that of simple pressure ascites.

Runeberg,[3] from the examination of 121 cases, arrives at the following general results :—

	Percentage of Proteid
In cases of hydræmia (including nephritis) the ascitic fluid contains	0·03-0·41
,, ,, portal obstruction ,, ,, ,,	0·37-2·68
,, ,, general venous congestion (heart disease) ,,	0·84-2·3
,, ,, carcinomatous peritonitis ,, ,,	2·7 - 3·51

For diagnostic purposes it may roughly be said that a high percentage of proteid denotes inflammation ; a low percentage of proteid certainly denotes absence of inflammation. The relation of albumin to globulin (proteid-quotient) is of no diagnostic value, as it varies with the proteid quotient of the blood ; it is therefore merely of theoretical interest.

There is generally a small percentage of sugar in the peritoneal fluid as in lymph generally. Many cases of cirrhosis of the liver, however, are often associated with a small amount of diabetes,[4] and the sugar in the ascitic fluid is thus increased. In one case of syphilitic cirrhosis of the liver, in which I examined the ascitic fluid, there was as much as 0·233 per cent. of sugar present. In a case of cirrhosis recorded by Moscatelli,[1] the percentage of sugar in the ascitic fluid was 0·15 ; this fluid also contained a small amount of allantoin. Sugar was, however, in this last case absent from the urine. It is only when the percentage of sugar in blood and lymph exceeds 0·2 per cent. that glycosuria ensues.

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[1] In addition to this, the fibrinogen was in the fourth tapping estimated by adding fibrin-ferment and weighing the fibrin formed; it amounted to 0·075 per cent. The above analysis is my own.

[2] J. J. Pigeaud, *Maly's Jahresbericht*, xvi. 474.

[3] Runeberg, *Deutsch. Arch. f. klin. Med.* xxxiv. 1. *See* also Hofmann (*Virchow's Arch.* lxxiii. 250) for a large number of similar analyses.

[4] Cobrat, 'De la glycosurie dans les cas d'obstruction partielle ou totale de la veine

Chylous ascites.—There have been several cases published of disease affecting the thoracic duct, and causing its rupture ; this leads to the extravasation of the chyle into the peritoneal cavity, and the fluid may be removed by tapping. I here merely quote two cases to illustrate this : Case I.[2] was a case published by Whitla, in which tuberculous disease led to the rupture of the duct in a boy of 13 ; Case II.[3] is published by J. Strauss, and was a case in which cancerous growths led to the rupture of the mesenteric lacteals. The analysis in the first case was made by Matthew Hay, in the second case by Guinochet.

	Case I	*Case II*
Constituents	Parts per 1000	
Fat	10·30	9·48
Proteids	28·78	21·08
Other organic matters	8·02	11·685
Mineral salts	9·95	1·595
Loss	—	0·510
Total Solids	59·15	43·795

Hay found a small percentage of sugar in the liquid ; Guinochet found neither sugar nor peptone.

In some cases of ascites, the fluid though not chylous yet contains a large excess of cholesterin, crystals of which are to be seen floating about in the liquid ; in other cases, the peculiar mucin-like substances paralbumin and metalbumin (which are pretty constant constituents of ovarian fluid, and will be described with that fluid) may be found. In still another class of cases of ascites, hæmorrhage may occur into the peritoneum, and a liquid more or less stained with blood or altered blood pigment is obtained on paracentesis (tapping).

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porte,' *Lyon. méd.* 1875, No. 15. Lépine, *Gaz. méd. de Paris*, 1876, p. 123. Quincke, *Berl. klin. Wochenschrift*, 1876, No. 38.

[1] Moscatelli, *Zeitschr. f. physiol. Chemie*, xiii. 202.

[2] *British Medical Journ.* vol. i. 1885, p. 1089.

[3] *Arch. de physiol.* xviii. 367. Another case is recorded by Maguire, *Brit. Med. Journ.* vol. ii. 1886, p. 197.

PLEURAL FLUID

The fluid has the same general characteristics as peritoneal fluid. It as a rule contains more proteids than the peritoneal fluid. It does not readily coagulate spontaneously, unless pleurisy be the cause of the exudation. A few more analytical data, in addition to those which have been already given, may be added as illustrations to the following propositions.

(1) In hydrothorax, the total percentage of proteid is much lower than in cases of pleurisy: the amount of fibrinogen as estimated by the weight of fibrin formed[1] is also less in the fluid of hydrothorax.

The following numbers are obtained from analyses of my own :—

Case	Specific Gravity	Total Proteids per cent.	Fibrin	Serum-Globulin	Serum-Albumin
1. Pleurisy (acute) .	1023	5·132	0·016	3·002	2·114
2. Pleurisy (acute) .	1020	3·4371	0·0171	1·2406	1·1895
3. Pleurisy (acute) .	1020	5·2018	0·1088	1·760	3·330
4. Hydrothorax (Bright's disease)	1015	2·5183	0·0067	0·6597	1·8519
5. Hydrothorax (Bright's disease)	1012	1·3242	0·0062	0·4026	0·9154
6. Hydrothorax (heart disease)	1016	1·482	0·013	0·779	0·700

(2) In hydrothorax, as in ascites, the liquid removed by successive tappings remains fairly constant in composition.[2]

Constituents	1st Tapping	2nd Tapping
Water	966·24	963·95
Solids	33·76	36·05
Organic solids	26·12	28·50
Inorganic solids	7·64	7·55

Sugar seems to be fairly constantly present in pleuritic fluid, as in other forms of lymph. In 17 specimens examined by H. Eichhorst[3] 10 contained small quantities of sugar.

Exceptional forms of pleuritic effusion are sometimes found ; some are associated with carcinomatous or sarcomatous tumours, and the cells characteristic of these growths may be found in the pleural liquid, in addition to the usual leucocytes. In other cases hæmorrhage may occur into the pleura. In one case of hæmorrhagic pleurisy,[1] I found a large amount of cholesterin floating about in a crystalline form in the liquid. In another case (not hæmorrhagic) there were large corpuscles like Gluge's inflammatory corpuscles[2] in large numbers, in addition to leucocytes.

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[1] In the case of the liquid of hydrothorax fibrin may be formed by adding serum- or fibrin-ferment to the liquid. The fluid in chronic pleurisy closely resembles that of hydrothorax (C. Méhu, *Bulletin Méd. du Nord*, 1872).

[2] The example selected is an analysis by C. Schmidt. It and others will be found on pp. 602–3 of Hoppe-Seyler's *Physiol. Chemie*.

[3] *Zeitschrift f. klin. Med.* iii. 537.

Cases of chylous pleurisy have also been described.[3]

PERICARDIAL FLUID

This fluid is not so often removed from the human subject in cases of disease when it is present in excess, as in other forms of dropsy, because of the greater danger attending the operation.

It is stated to contain a larger quantity of fibrinogen than other transudations, and Kühne found that it contains 0·879 to 2·468 per cent. of proteids.

Dr. Friend has made under my superintendence the following analyses of the pericardial fluid of the horse removed after death.

In parts per 1000	I	II
Water	964·011	957·953
Solids	35·989	42·047
Proteids	28·641	25·846
Fibrinogen (estimated as fibrin)	0·117	0·260
Serum-globulin	11·069	11·603
Serum-albumin	17·455	13·983
Extractives	—	2·432
Salts	7·575	13·769
Specific gravity	1018	1018
Reaction	alkaline	alkaline

It is interesting to note that the pericardial fluid of the tortoise, which I have examined, exhibits precisely the same characters and properties as that of the mammalian animals.

Chylous effusions into the pericardium may occur, as in the case of the other serous sacs; a case is recorded by K. Hasebroek,[4] and it may be useful to compare the analysis of the fluid he obtained with those of non-chylous pericardial fluid as recorded by previous observers. The chylous fluid will be seen to contain a greater amount both of proteids and extractives than ordinary pericardial fluid : more than 50 per cent. of the extractives consisted of fat.

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[1] Under Dr. Ringer's charge, Univ. Coll. Hosp.

[2] Corpuscles three or four times the size of white blood corpuscles, containing numerous fat granules.

[3] For the analysis of one *see* Hoppe-Seyler's *Physiol. Chem.* p. 596.

[4] *Zeit. physiol. Chem.* xii. 289.

In parts per 1000	1. Chylo-pericardial Fluid (Hasebroek)	2. (Gorup-Besanez)[1]	3. (Wachsmuth)[2]	4. (Hoppe-Seyler)[3]
Water	892·782	955·1	962·5	961·78
Solids	103·612	44·9	37·5	38·22
Fibrin	—	0·8	—	—
Proteids	73·789	24·7	22·8	24·63
Extractives . . .	20·481	12·7	—	—
Salts	9·336	6·7	—	—

HYDROCELE FLUID

This fluid with the preceding (pericardial fluid) is interesting historically ; these fluids having been very largely employed in the course of experiments on the coagulation of the blood (*see* p. 243). It is contained in the tunica vaginalis, originally a part of the peritoneum, and is itself almost exactly like the peritoneal fluid. It resembles the peritoneal fluid and other forms of lymph in reaction, colour, and constituents. It does not as a rule clot spontaneously, unless mixed with blood or serum, or containing an excess of leucocytes from inflammation. Its specific gravity varies from 1016 to 1022 ; the amount of proteid present also varies very much. The following is the mean of 17 analyses made by Hammarsten :[4]—

Water	938·85	parts	per 1000
Solids	61·15	,,	,,
Fibrin	0·59	,,	,,
Globulin	13·52	,,	,,
Albumin	35·94	,,	,,
Ether extractives . . .	4·02	,,	,,
Salts	9·26	,,	,,
NaCl	6·19	,,	,,

There are cases of hydrocele which differ from the ordinary fluid ; some are viscous from the presence of metalbumin and paralbumin[5] (*see* Ovarian Fluid) ; some contain excess of cholesterin ; and others are chylous. The cases of chylous hydrocele are sometimes associated with chyluria (chylous urine); and chyluria is produced by the presence of the hæmatozoon *Filaria Sanguinis Hominis* in the blood (*see* Urine). Lymph tumours and tumours filled with chyle are very common in the scrotum and its neighbourhood in cases of chyluria, and these may discharge their contents from the surface of the skin.[1]

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[1] *Lehrbuch*, p. 401.

[2] *Arch. f. pathol. Anat.* vii. 334.

[3] *Physiol. Chemie*, p. 605.

[4] Quoted from Hoppe-Seyler's *Physiol. Chemie*, p. 606 (Hammarsten's original paper s in Swedish). Other analyses by Hoppe-Seyler will be found on the same page.

[5] R. Devillard, *Bull. soc. chim.* xlii. 617.

Other cases of chylous hydrocele may however occur, which seem to be produced like chylous ascites by the rupture of lacteals. I have examined one such case; it was a fluid obtained from a case of otherwise ordinary hydrocele, which was shown to the Pathological Society by Mr. S. G. Shattock.[2]

THE FLUID OF SUBCUTANEOUS ŒDEMA

This fluid is poorest of all the dropsical fluids in proteid constituents; otherwise it resembles them very closely.

Some analyses have already been given of this fluid, in the comparisons that we have drawn between it and other effusions; the following are some estimations of the proteid constituents which I have made.

Case	Specific Gravity	Total Proteids per cent.	Fibrin	Serum-Globulin	Serum-Albumin
1. Cardiac dropsy; fluid from incisions in ankles . . .	1012	0·33	0·0028	—	—
2. Cardiac dropsy, another case; fluid obtained in the same way.	1013	0·592	traces	0·139	0·453
3. Bright's disease; fluid removed by Southey's trocar and cannula from ankles .	1009	0·6404	traces	0·1911	0·4493

In all these cases the fluid which drained away first, coagulated spontaneously on standing; this was due to a slight admixture with blood. The fluid collected after hæmorrhage had ceased did not coagulate spontaneously, but on adding blood or serum to it, a small quantity of fibrin was in all cases obtainable.

A. Rosenbach[3] has made a special investigation whether sugar is present or not, and he finds that it is nearly constantly present in œdema fluid in small quantities.

Blister fluid.—This fluid has the same relation to œdema fluid, as that of peritonitis or pleuritis to that of simple pressure dropsies into the serous cavities. It contains a large number of leucocytes, coagulates spontaneously when drawn, has a higher specific gravity (1018 or more), and a larger percentage of proteids, as is seen by boiling it, when it becomes almost solid from the heat-coagulum produced.

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[1] Analyses of such fluids will be found in Hoppe-Seyler's *Physiol. Chem.* pp. 608–9.

[2] The case was one tapped by Sir Henry Thompson, see *Trans. Path. Society*, xxxv. 250. Mr. Shattock informs me he has since seen a case similar to that which I analysed for him.

[3] A. Rosenbach, *Breslauer ärztl. Zeit.* 1885. No. 5.

In cases of gout, blister fluid like the blood plasma contains excess of urates, and the method of examining it for uric acid, as originally suggested by Garrod, has been already described (p. 252).

THE AQUEOUS HUMOUR

The anterior chamber of the eye is essentially a lymph space, and the fluid in it, the aqueous humour, is essentially lymph; but lymph which contains a very small proportion of proteid constituents. The amount of aqueous humour is directly dependent on the blood pressure (Chavvas).[1]

Lohmeyer[2] analysed the aqueous humour of the calf, and the following are his results in parts per 1000 :—

Water	986·87
Solids	13·13
Proteids	1·22
Extractives	4·21
Inorganic salts	7·70
	(Sodium chloride 6·89)

The aqueous humour either does not coagulate spontaneously, or clots very slowly; it contains in health no formed elements. As in other forms of lymph, however, a clot of fibrin is formed on the addition of serum. The proteids are the same in kind as in blood plasma and lymph generally, viz. fibrinogen, serum-globulin, and serum-albumin.[3]

Kuhn[4] finds among the extractives that a reducing substance like sugar is constantly present in the aqueous humour of the ox and rabbit; the percentage of this substance reckoned as dextrose, present in the aqueous humour of the ox, is 0·03–0·04. This substance is not sugar, as it will not undergo the alcoholic fermentation (Gruenhagen).[5]

Urea and sarcolactic acid (Gruenhagen) are also present in small quantities.

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[1] Chavvas, *Pflüger's Archiv*, xvi. 143.

[2] *See* Gorup-Besanez, *Lehrbuch*, 4th edit. 1878, p. 401.

[3] Friend and Halliburton, *Brit. Ass. Reports*, 1889, p. 130.

[4] Kuhn, *Pflüger's Archiv*, xli. 200.

[5] Gruenhagen, *ibid.* xliii. 377.

PERILYMPH AND ENDOLYMPH

These fluids of the internal ear have been examined by Dähnhardt[1] in fishes. Perilymph contains 2·1–2·2 per cent. of solids; it is rich in mucin and in sodium chloride. The endolymph is clearer, less viscid; it contains 1·5 per cent. of solids, including a small quantity of mucin.

SYNOVIA

The fluid in synovial cavities around joints, in bursæ, sheaths of tendons, &c., differs from that in serous cavities (1) in containing a greater proportion of solids; (2) in containing a slimy mucin-like substance which confers viscosity upon it.

The following are the analyses that have been published.

In parts per 1000	Synovia of Calf (Frerichs)[2]	Synovia of Ox. Mean of Two Analyses (Frerichs)[2]	Synovia from knee of a Man in which there was excess (Hoppe Seyler)[3]	Human Synovia from two similar cases (Hammarsten)[4]	
				Case 1, Chronic	Case 2, Acute
Water	965·7	959·2	928·33	947·19	933·7
Solids	34·3	40·8	71·67	52·81	66·3
Mucin	3·2	4·0	6·6	2·7	3·56
Proteids.	19·6	26·05	51·3	39·2	54·21
Extractives, fat, &c.			4·47	4·96	3·5
Mineral salts . . .	10·6	10·6	9·3	8·65	8·53 (NaCl 6·26)

Frerichs found that active exercise diminishes the amount, and increases the concentration of synovia.

Some doubt has arisen as to whether the slimy substance present in synovia is really mucin. In Hammarsten's two cases there was no true mucin, but the slimy substance was found to be nucleo-albumin, like that which causes the sliminess of bile; it contained 5 per cent. of phosphorus. Landwehr[5] on the other hand maintains that the slimy substance in synovia is true mucin, i.e. a compound (or mixture) of a proteid with a carbohydrate called animal gum.

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[1] Dähnhardt, *Arbeit d. Kieler physiol. Inst.* p. 103.

[2] Frerichs, R. Wagner's *Handwörterb. d. Physiol.* iii. 463.

[3] Hoppe-Seyler, *Physiol. Chem.* p. 623. In other similar cases Hoppe-Seyler found 10·91 per 1000 of mucin.

[4] Hammarsten, *Maly's Jahresbericht*, xii. 484; original paper in Swedish.

[5] Landwehr, *Pflüger's Archiv*, xxxix. 193.

THE FLUID IN OVARIAN CYSTS

The fluid contained in ovarian cysts has for its basis a transudation from the blood vessels, as in the different forms of dropsy into serous cavities; it is, however, generally viscous, from the presence of a mucinoid material, which masks the other proteid constituents of the fluid.

Ovarian fluid is alkaline; it is often coloured with a deep brown pigment, derived in all probability from hæmoglobin, and often contains excess of cholesterin, crystals of which are seen floating about.

Oerum [1] has examined numerous specimens of fluid obtained from various forms of ovarian cysts, and his general analytical conclusions may be thus summarised:—

Colloid Cystomata (24 *Cases*)

Specific Gravity	Total Solids	Salts	Proteids, including mucin-like substance
Maximum, 1038 Minimum, 1010 In four cases only, above 1030	In 13 cases 25–75 parts per 1000 In 2 cases less than 25, and in 1 case more than 75 per 1000	6–8·7 parts per 1000	Maximum, 108·32; minimum, 8·8 per 1000. In only three cases greater than 50 per 1000 Fibrin was formed on mixing the fluid with blood. Peptone absent. Mucin-like substance always present
Papillary Cystomata.—2 Cases			
1036	116·4 parts per 1000	—	102·67 parts per 1000. In one case the mucin-like substance was present, in the other absent
Hydrops Folliculi Graafiani.—2 Cases			
1009	—	—	Fluid clear and watery. Mucinoid substance absent.
Parovarial Cystomata			
—	Small in quantity	—	Clear watery fluid. Mucinoid substance absent. (In one case I had the opportunity of examining I found 1 per cent. of proteids[2])
Hydrops Tubæ.—2 Cases			
1008	10·5–11 per 1000	6–7 per 1000	1·2 per 1000. No mucinoid substance
Fluid from Fibro-Cystic Tumour.—1 Case			
—	—	—	63·056 per 1000, of which 3·58 per 1000 consisted of fibrin, the remainder serum-globulin and serum-albumin. No mucinoid substance present

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[1] Oerum, 'Kemiske Studier over Ovariecystevaedsker, &c.' Koebenavn, 1884, *Maly's Jahresbericht*, xiv. 459.

[2] *Journal of Physiology*, v. 163.

We have here a large number of fluids from various diseases of the ovary and neighbouring organs; and we see that the chief point of interest is the constant presence of the mucinoid material in colloid cysts, and this is regarded by Oerum as being diagnostic of the colloid form of degeneration. It is not present in the normal ovary, nor in cases of hydrops of the graafian follicles. In those cases of ascites where the same material occurs, colloid degeneration is also present.

This peculiar ropy, slimy material was by Scherer said to consist of two substances, which he named metalbumin and paralbumin. Both can be precipitated by means of alcohol, and the alcoholic precipitate is easily soluble in water. Metalbumin is the name given to the ropy substance, and paralbumin to the substance which occurs in colourless ovarian fluids which have a gummy consistency.

These substances have been investigated by Hammarsten,[1] whose results are briefly as follows:—

Metalbumin is simply colloid material, i.e. the substance formed in colloid degeneration. It has the physical characters of mucin; chemically, like mucin, it yields a reducing sugar on boiling with dilute sulphuric acid; it, however, is not mucin, as it is not precipitable by acetic acid; the name pseudo-mucin is therefore suggested. *Paralbumin* is simply pseudo-mucin in loose combination with a proteid.

These facts have been fully confirmed by Landwehr[2]; he regards these substances as belonging distinctly to the class of mucins; they contain a carbohydrate called animal gum, and it is this which is converted into a reducing sugar by the action of dilute sulphuric acid.

In one case of ovarian fluid, which I very fully examined,[3] the fluid was opalescent but not at all viscous. On adding acetic acid there was an abundant precipitate. On examination, this precipitate was found to consist of true mucin.

THE FLUID IN HYDRONEPHROSIS

The ureter of one kidney may become blocked by a stone or new growth; that kidney becomes functionless, while the other does double work. The functionless kidney becomes more and more filled with urine, and dilates, till ultimately a large sac containing dilute urine is formed, and into it some proteids from the blood also pass. The urinary constituents in time are absorbed. The following details of an analysis (Oerum, *loc. cit.*) may serve as an example:—

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[1] *Maly's Jahresbericht*, xi. 11. Original paper in Swedish.

[2] A. Landwehr, *Zeit. physiol. Chem.* vii. 118.

[3] *Brit. Med. Journ.* vol. ii. 1890, p. 196.

Specific gravity, 1009.
Solids, 20·441 per 1000.
Proteids, 7·677 „ „ (serum-globulin and serum-albumin).
Salts, 8·654.
Urea, uric acid, creatinine, absent.

The fluid, in other words, resembles a serous effusion.

Cystic degeneration of the kidney may occur from causes which are not known, and consists in the formation of small cysts throughout the kidney substance; the contents of these cysts are watery, sometimes contain urinary constituents, sometimes are tinged with blood, and sometimes, i.e. when associated with colloid disease, are viscous.

THE FLUID IN HYDATID CYSTS

The fluid which accumulates in the interior of the cysts formed by the parasite *Tænia echinococcus* is colourless, neutral, and sometimes slightly opalescent. Its specific gravity is 1006-1015; it contains 1·2 to 1·4 per cent. of solids. The solids may be classified in the usual way into proteids, which are very scanty, extractives (among which sugar and inosite, and traces of urea, creatine and succinic acid have been described), and salts.

Microscopically it is often seen to contain a number of hooklets from the embryos. If allowed to escape into the peritoneal cavity, it is stated that it sets up peritonitis; in connection with this point it may be noted that Mourson and Schlagdenhauffen[1] have found a poisonous ptomaine in the liquid.

If the growth has involved organs producing the rupture of blood vessels, or bile vessels, the fluid obtained will be mixed with blood or bile respectively. In one case which I examined the fluid consisted of little else but bile.

THE AMNIOTIC FLUID

The most probable suggestion as to the origin of the amniotic fluid, during the early months of pregnancy, is that it is simply exuded from the tissues of the fœtus. After the formation of the placenta, and chorionic vessels, a transudation of lymph takes place from the maternal vessels into the amniotic cavity.[2] In the later months of pregnancy this becomes mixed with fœtal urine.[3] The composition of the fluid corresponds to its double origin; it contains, in addition to water, oteids, urea, and the salts common to urine and blood. Its quantity ries between one and two pints. In some cases it is abnormally nall in quantity, in others (hydramnion) abnormally large.

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[1] *Compt. rend.* xcv. 791.

[2] Jungbluth, 'Beitrag zur Lehre vom Fruchtwasser,' *Inaug. Dissert.* Bonn, 1869.

[3] Gusserow, *Arch. f. Gynäk.* iii. 268. Prochownik, *Ibid.* xi. 304.

A comparison of normal amniotic fluid with that of hydramnion as been made by Prochownik, who gives the following numbers :—

Parts per 1000	Amniotic Fluid	Fluid of Hydramnion
Water	984·3	981·4
Solids	15·7	18·6
Proteids	1·9	5·2
Extractives	8·1	7·7
Salts	5·9	5·6

In hydramnion the proteids are thus more abundant than normal. Scherer[1] and Weyl[2] found mucin in amniotic fluid.

CEREBRO-SPINAL FLUID

This is the name given to the fluid which is present in the cerebro-spinal cavity, that is, the ventricles of the brain, and the central canal of the spinal cord; the same fluid is also present outside the spinal cord in the subarachnoid and subdural cavities; the communication between the fluid outside and inside the central nervous system is by means of the foramen of Majendie, a hole which perforates the piece of pia mater which forms part of the roof of the fourth ventricle.

By some previous observers the cerebro-spinal fluid has been regarded simply as an exudation from the blood, and has been classified with the fluids which occur in serous cavities. This is, however, incorrect because :—

1. The arachnoid membrane is neither from the point of view of embryology or structure a serous membrane.
2. The fluid is not a mere lymph moistening the parts already enumerated, but is normally present in sufficient quantity to exercise a considerable amount of pressure.
3. Chemical investigation of the fluid itself shows that it is very different from the fluids contained in serous membranes, and thus support is lent to the idea originally propounded by C. Schmidt, that the fluid should be classified rather with secretions than with transudations.

The normal cerebro-spinal fluid is obtained in cases of fracture of the base of the skull, where sometimes the fluid escapes by the ear, if the membrana tympani has been also ruptured by the accident.

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[1] Scherer, *Zeit. f. wiss. Zool.* i. 89.

[2] Weyl, *Arch. f. Anat. u. Physiol.* 1876, p. 543.

In some cases of congenital deficiency of the vertebral arches (spina bifida), a tumour of the membranes of the spinal cord (meningocele) projects through the opening, and in this the fluid accumulates, and may be removed by tapping. This fluid may be also regarded (or at least that obtained by the first tapping) as normal cerebro-spinal fluid.

In the disease known as hydrocephalus excess of the cerebro-spinal fluid accumulates within the ventricles of the brain, in some cases so much so that the brain becomes a mere sac surrounding the fluid. Hydrocephalus may be chronic or acute. Chronic hydrocephalus is due to an accumulation of the normal fluid; it is often associated with pressure upon, or obstruction in, the veins at the base of the skull, and in these cases, the fluid is therefore cerebro-spinal fluid *plus* a transudation from the blood. In acute cases (tubercular meningitis) the specific gravity rises, and the solid constituents also increase, especially the proteids; and in such cases the fluid resembles the exudations which occur in inflammations elsewhere. It is, however, rare for the fluid to become purulent.

In animals small quantities of the fluid may be obtained by means of a small syringe from beneath the dura mater.

Necessarily the greater part of our knowledge of the fluid from the central nervous cavity is derived from pathological cases where the fluid is in excess.

The liquid is always either neutral or faintly alkaline, and has a specific gravity of about 1007-8.

Composition.—The following analyses of the fluid from spina bifida were made by myself:—[1]

	Case 1. Female æt. 19 In parts per 1000	Case 2. Child æt. 11 days First Tapping In parts per 1000	Case 3. Child æt. 13 weeks Fourth Tapping In parts per 1000
Water	989·75	989·877	991·658
Solids	10·25	10·123	8·342
Proteids	0·842	1·602	0·199
Extractives . . .	9·626	0·631	3·028
Salts		7·890	5·115

The following analyses of the fluid from cases of chronic hydrocephalus are from C. Schmidt. They show in comparison with spina bifida fluid rather a greater quantity of solids, especially of proteids.

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[1] 'Report of Spina Bifida Committee,' vol. xviii. of the *Clin. Soc. Trans.* Similar analyses will be found in Hoppe-Seyler's *Physiol. Chemie*, p. 604.

In parts per 1000	Case 1	Case 2	Case 3
Water	986·78	984·59	980·77
Solids	13·22	15·41	19·23
Proteids and Extractives .	3·74	6·49	11·35
Salts	9·48	8·92	7·88

A tabular statement, such as the preceding, does not show, however, that there is anything characteristic in the fluid. It is when we come to examine the various constituents of the fluid that we find how it differs from the fluids in serous sacs. These differences consist in the presence of certain peculiar proteids, and of a substance which reduces copper salts like sugar.

Proteids.—The proteids in normal cerebro-spinal fluid (removed from cases of meningocele) are as follows :—

(*a*) Fibrinogen is absent. There is no heat-coagulum produced by a temperature of 56°C. No fibrin is formed on the addition of serum or of fibrin-ferment.

(*b*) All the proteid present is precipitable by saturation with magnesium sulphate. Serum-albumin is therefore absent. Hoppe-Seyler[1] describes the proteid which is present as a globulin. On redissolving the precipitate, however, it is found on heating the solution that a very small heat-coagulum is found at 75°C.,[2] but that the remaining proteid consists of proteoses or albumoses, i.e. proteids like those which are formed during digestion, intermediate bodies in the formation of peptone. There, however, appears to be no proteolytic ferment, like pepsin or trypsin, present in the fluid. The most characteristic properties of albumoses are :—

i. They are not coagulated by heat.

ii. They are precipitated by nitric acid in the cold ; the precipitate disappears on heating, and falls down again on cooling.

iii. Like peptones they give a pink colour with copper sulphate and caustic potash ; other proteids give a violet colour.

The form of albumose most frequent in cerebro-spinal fluid is proto-albumose, i.e. one which is precipitable by saturation with sodium chloride or magnesium sulphate. In some few cases deutero-albumose has been found, i.e. one which is not precipitable by the salts just mentioned; it is, however, precipitated by saturation with ammonium sulphate; and in other cases still fewer in number, true peptone is found, i.e. a proteid which is not precipitable by saturation with ammonium sulphate.

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[1] Hoppe-Seyler, *Physiol. Chemie*, p. 608.

[2] This temperature is the same as that at which serum-globulin is coagulated. The globulin present appears to be serum-globulin. Cell-globulin (fibrin-ferment) is absent.

The existence of albumoses in cerebro-spinal fluid may be shown another way. A large excess of alcohol is added to the fluid; this precipitates all the proteids; and after a few weeks the globulin is rendered insoluble in water by the action of the alcohol. The albumoses, however, remain still freely soluble.[1]

In cases of chronic hydrocephalus, the fluid removed by the first tapping has the normal characteristics of cerebro-spinal fluid. The fluid removed by subsequent tappings, however, resembles a dilute transudation from the blood; and if inflammation supervenes, this becomes more marked. Albumose can still be shown to be present, but it is obscured by the superabundance of the other proteids. An increase in the amount of proteids in successive tappings does not necessarily occur either in spina bifida or hydrocephalus; but in the latter disease, an increase is very apt to occur, perhaps as a result of irritation, produced by the removal of the fluid; the quantity does not, however, rise so high as it does in cases of acute, i.e. inflammatory, hydrocephalus. At the same time the reducing substance also becomes more abundant. The following case of chronic hydrocephalus in a boy six months old, under the care of Mr. Parker,[2] and of which I had the opportunity of examining the fluid in three successive tappings, illustrates this point very well.

	Specific Gravity	Proteids		Reducing Substance
		Percentage of Total Proteids	Kinds of Proteid present	
First Tapping .	1006	0·045	Globulin Proto-albumose Hetero-albumose	Traces
Second „ .	1010	0·069	The same as in the first tapping	Fairly abundant
Third „ .	1010	0·272	Serum-globulin Serum-albumin Trace of albumoses	More abundant

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[1] I have examined a number of specimens of blood and transudation for albumoses and peptone, but in all cases with a negative result (*Proc. Physiol. Soc.* 1887, p. xiv. *See* also *J. Physiol.* vol. x. p. 232).

[2] East London Hospital for Children.

In the fluid from a case of acute hydrocephalus,[1] the following details of the analysis show very well the characteristics of this fluid :—

The fluid was clear, alkaline, and straw-coloured ; the colour was extracted by alcohol, and had the normal characteristics of serum-lutein.

Proteids : the percentage present was 0·65. The fluid contained a small clot of fibrin. Magnesium sulphate produced an abundant precipitate of serum-globulin ; serum-albumin remaining in solution. The presence of albumoses was questionable.

Reducing substance : present in small quantities. In other words, the fluid in cases of acute hydrocephalus resembles other inflammatory exudations in the higher percentage of total proteids, and in the presence of fibrinogen, and white blood corpuscles, which caused clotting when the fluid was removed from the body.

Reducing substance.—It has long been known that cerebro-spinal fluid contains a substance which reduces copper salts in the same way as sugar does. Bussy[2] found it in the fluid obtained from a patient with a fractured cranium, and in the cerebro-spinal fluid of the horse and dog ; but although he considered it was grape sugar that was present, he found he was not able to induce alcoholic fermentation in it. Turner[3] discovered the same substance in spina bifida fluid, but he also found that the addition of yeast produced no fermentation ; he concluded that the reduction is brought about, not by a carbohydrate, but by some derivative of albumin. Since then it has been abundantly shown that the substance is not sugar ; it does not reduce bismuth salts ; it does not rotate the plane of polarised light ; it does not form a crystalline compound with phenylhydrazine as sugar does. It is thus not sugar. The substance is pyrocatechin (catechol) ; and it may be separated in the following way : alcohol is added to precipitate the proteids ; the alcoholic extract is evaporated to dryness, and the residue dissolved in water ; neutral lead acetate is added to the aqueous solution ; a precipitate is formed, this is suspended in distilled water, and the lead separated by a stream of sulphuretted hydrogen, and filtered. The filtrate is shaken with ether ; the ethereal extract on evaporation yields a crystalline deposit which consists of pyrocatechin. This is a substance of which the formula is $C_6H_6O_2$; it belongs to the aromatic group of organic compounds. It is turned green by ferric chloride, brown by caustic alkalis ; it has a peculiar pungent taste and an acid reaction. It is one of the products of decomposition of proteids, and occasionally is found in urine (*see* p. 77).

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[1] The fluid was sent to me by Dr. Penrose ; it was removed a few days before death from a boy 5 months old, in the Hospital for Children, Great Ormond Street.

[2] *Bulletin de l'académie de médecine*, Dec. 1852.

[3] W. Turner, *Proc. Roy. Soc.* vol. vii. (1854), p. 89.

This substance appears to be a normal constituent of cerebro-spinal fluid, though whether in a combined or an uncombined state is at present doubtful. Hoppe-Seyler states that the reducing substance only occurs after irritation has been set up by tapping; but this does not appear to be the case. I have never failed to find it in fluid removed by the first tapping, though it may occasionally be so scanty that the fluid must be concentrated before it can be discovered. In subsequent tappings it is always increased in quantity. In one case (first tapping) the quantity (reckoned in terms of dextrose) was 0·002 per cent.; in another case (fourth tapping) the percentage was 0·165.

Salts.—Carl Schmidt remarked that his analyses of the inorganic constituents of cerebro-spinal fluid showed an unusual preponderance of potassium salts. The following are the numbers:

Parts per 1000	Case 1 Hydrocephalus	Case 2 Hydrocephalus	Case 3 Hydrocephalus
K_2SO_4	0·096	0·193	0·222
KCl	2·181	1·485	0·232
NaCl	4·438	4·101	6·054
Na_3PO_4 . . .	0·613	0·486	0·115
Na_2O	1·842	2·290	0·987
$Ca_3(PO_4)_2$ . . . $Mg_3(PO_4)_2$ . . .	0·307	0·362	0·271

Two of these cases certainly show a remarkably high percentage of potassium chloride; but subsequent investigators have not found that this is a general rule. Yvon[1] gives the following numbers; the fluid was removed from a case of hydrocephalus.

NaCl	7·098	per 1000
KCl	0·033	"
CaO	0·112	"
P_2O_5	0·563	"
SO_3	traces	
Iron	traces	
Magnesia	0·238	"

F. Müller[2] in another case found that the inorganic salts present were 8·8 per 1000. The most abundant salt present was sodium chloride, and the relation of NaCl to KCl was 21·5 to 1.

In my own experiments I sought to obviate error in making observations on the saline constituents of this fluid by avoiding incineration; there is no doubt that some of the salts, especially sodium chloride, pass off with the organic matter during the process of ignition. It is also well to take a large quantity of fluid so that errors may be minimised. The following method may be recommended for determining the relation of sodium and potassium in organic liquids.

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[1] Yvon, *Journ. de pharmacie et de chimie*, 4th series, vol. xxvi. (1877), p. 240.

[2] F. Müller, *Mittheil. a. d. Würzburger med. Klinik*, i. 267.

The liquid is first evaporated to dryness, and the organic matter is destroyed by heating with fuming nitric acid ; the residue is evaporated to dryness two or three times on the water-bath with hydrochloric acid, in order to convert all sodium and potassium compounds into chlorides ; phosphates, lime, and magnesia are precipitated by making the liquid just alkaline with baryta water, and the precipitate so formed is filtered off. Excess of baryta is then precipitated with ammonium carbonate, and filtered off ; the residue is evaporated to dryness on a weighed platinum capsule, and the increase in weight gives the total chlorides ; these are dissolved in water, and platinum chloride added ; this precipitates the potassium chloride, and from the weight of the precipitate the potassium chloride can be calculated ; the difference between total chlorides and potassium chloride gives the amount of sodium chloride.

The following numbers were obtained in one analysis of hydrocephalus fluid : 300 c.c. of fluid yielded 2·7825 grammes of chlorides, i.e. 0·927 per cent. The weight of potassium chloride calculated from the weight of the platinum precipitate was 0·0859 gramme, or 0·028 per cent. Therefore in 100 parts of chlorides 4·85 consisted of potassium chloride and 95·15 of sodium chloride. This is about the same proportion as is present in blood, lymph, and transudations generally.

PUS

Pus is the creamy fluid which occurs in abscesses. We have already seen how in inflammation the normal transudations from the blood vessels become increased in amount, richer in solids, and in corpuscular elements. The emigration of the white corpuscles may go on to such a great extent that they crowd in great numbers in the exuded liquid ; in fact, this is the process known as suppuration or pus formation.

The microscopical appearances of pus are as follows :—It is a clear fluid crowded with leucocytes, which have undergone more or less degenerative change ; fat globules are also found which have been liberated from leucocytes that have undergone fatty degeneration and burst. In pus also one usually finds abundant micro-organisms (micrococci and bacteria). The cells of the pus are called pus-corpuscles, the liquid in which they float the pus-serum. The specific gravity of pus is 1030–1040, and its reaction is alkaline.

Pus corpuscles.—There is no doubt that these are chiefly white blood corpuscles which have exuded from the vessels; some of the cells are probably, however, derived from the tissues in which the formation of the abscess is taking place (connective tissue corpuscles). The cells do not as a rule show any active amœboid movement. They are spherical, and swollen in many cases with fat globules, their protoplasm having undergone fatty degeneration. In some cases the pus cells have still further disintegrated, and may even have an acid reaction from the formation of sarco-lactic acid.

For chemical investigation pus cells may be obtained by mixing pus with an equal volume of dilute sodium sulphate solution (a saturated solution of the salt diluted with nine times its volume of water), and then filtering; the pus corpuscles remain on the filter, and are washed with some of the same saline solution.

The various substances found in the pus cells are like those which we have already described in the white blood corpuscles.

The nuclei consist of nuclein, a phosphorised albuminoid substance. It may be separated from the investing protoplasm by treating pus with artificial gastric juice; the nuclei remain undissolved. The nuclein of pus cells was investigated by Miescher,[1] and also by Hoppe-Seyler, whose somewhat conflicting analyses certainly seem to denote that nuclein is not a definite chemical individual. The nuclein of pus corpuscles in its general characters does not appear to be different from that contained in nuclei elsewhere (p. 202).

The protoplasm consists of proteids chiefly, but it also contains various extractives and a certain small proportion of inorganic salts.

The following tabular statement gives the results of Hoppe-Seyler's analyses in two samples of pus-cells.

Organic constituents in 100 parts of dried pus-cells:—

	(1)		(2)
Proteids	13·762	68·585	67·369
Nuclein	34·257		
Insoluble substances . . .	20·566		
Lecithin	14·383		7·564
Fats			7·500
Cholesterin	7·400		7·283
Cerebrin	5·199		10·284
Extractives	4·433		

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[1] Miescher, 'Ueber die chemische Zusammensetzung der Eiterzellen,' Hoppe-Seyler's *Med. Chem. Untersuchungen*, p. 441.

[2] Hoppe-Seyler, *Med. Chem. Untersuchungen*, p. 497. For this reference I am indebted to Gamgee, *Physiol. Chem.* p. 244.

Inorganic constituents in 100 parts of dried pus-corpuscles :—

$NaCl$	0·435
$Ca_3(PO_4)_2$	0·205
$Mg_3(PO_4)_2$	0·113
$Fe_2(PO_4)_2$	0·106
PO_4	0·916
Na	0·068
K	traces

Proteids.—My own observations coincide with those of Miescher as regards the absence of myosin (described by earlier observers). The most abundant proteid is the same nucleo-albumin already described in the white blood corpuscles; originally called hyaline substance by Rovida, and also noted by Miescher (*see* p. 260). With sodium chloride and magnesium sulphate it swells up into a slimy mass, and hence one has to use sodium sulphate in separating the pus-corpuscles from the pus-serum. Cell-globulin and cell-albumin are also present in pus-cells as in white blood corpuscles. Fibrin-ferment has been prepared from pus by Rauschenbach.[1] In addition to these, which are the normal proteid constituents of leucocytes, there are often found in addition considerable quantities of albumoses and peptone produced no doubt during the retrogressive metamorphosis of the corpuscles. It is very probable that the fever which accompanies suppurative processes is often, at any rate in part, produced by the entrance of these substances into the circulation.[2]

The other constituents of pus-cells have been already enumerated, and there is but little to be added concerning them. The large increase of fat and fat-like substances (lecithin, cholesterin, &c.) should be noted; the fatty degeneration, of which this is an indication, can also be seen by the microscope; free fatty acids may even be found in old pus, forming crystalline deposits. Glycogen can be often demonstrated in pus corpuscles, microchemically by the use of iodine which stains it deep brown (Ranvier[3]). It has also been separated in considerable quantity from pus-cells (Salomon[4]). In containing glycogen, pus-cells resemble white blood corpuscles.

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[1] *Inaug. Dissert.* Dorpat, 1883. *Maly's Jahresbericht*, xiii. 134.

[2] Dr. S. Martin, *Brit. Med. Journ.* vol. ii. 1890, p. 284. The original statements concerning the presence of peptones in pus were made by Eichwald (*Verhandl. d. phys. med. Gesellsch.* Würzburg, 1864, p. 335), and Hofmeister (*Zeit. physiol. Chem.* ii. 295). The method adopted by these observers was not, however, perfectly trustworthy. The only reliable method is that adopted by Martin. The pus was placed under excess of alcohol for many weeks, dried, and extracted with water. Albumoses and peptones alone went into solution, the other proteids of the pus having been coagulated by the alcohol. For an account of the effects of the injection of albumoses and peptones in raising the body temperature, i.e. producing fever, *see* Ott and Collmar, *Journal of Physiology*, viii. 218.

[3] *Progrès méd.* 1877, p. 422.

[4] *Deutsch emed. Wochenschr.* 1877, No. 35.

Pus-serum.—This may be separated from the corpuscles by the use of the centrifugal machine ; or it may be obtained diluted with sodium sulphate solution after filtering off the pus corpuscles in the manner already described.

The pus-serum is like blood-serum in composition ; it differs from lymph and other forms of exudation which we have considered in containing no fibrinogen, and consequently no fibrin is formed when the pus is removed from the body. It is, however, possible that fibrin may be formed within the abscess, and be subsequently dissolved and absorbed; perhaps in some cases this leads to the 'inspissation' of the pus, as in very severe cases of pericarditis and empyema (purulent pleurisy).

The proteids of pus-serum are serum-globulin and serum-albumin ; the extractives and salts are like those in the blood and lymph generally, except that lecithin appears to be more abundant, and leucine and tyrosine have been found (Hoppe-Seyler).

Hoppe-Seyler's analysis of pus-serum may be tabulated in the following way :—

In parts per 1000	(1)	(2)
Water	913·70	905·65
Solids	86·30	94·35
Organic solids	78·57	86·58
Proteids	62·23	77·21
Lecithin	1·50	0·56
Other organic matters . .	14·84	8·81
Inorganic solids	7·73 (NaCl 5·22)	7·77 (NaCl 5·39)

Pigments in pus.—When hæmorrhage occurs into an abscess the blood pigment more or less altered will be found, and in other cases bile pigments have been described. In other cases again, pigments due to the activity of certain micro-organisms are found (chromogenic bacteria) ; thus pyocyanin is a blue or rather violet pigment, produced by the growth of a bacillus. It is soluble in water, alcohol, chloroform, and ether, and crystallises from chloroform in prisms or rectangular plates. Pyoxanthose is a yellow pigment similarly produced, and often accompanies pyocyanin. When the two pigments are together, the pus appears green. Pyoxanthose differs from pyocyanin in its solubilities, and may be separated from the latter body by the use of ether in which it is the more soluble (Fordos,[1] Lücke,[2] Fitz,[3] Kunz,[4] Babès[5]).

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[1] *Comptes rend.* vol. li. (1860), p. 215.

[2] *Archiv f. klin. Chirurgie*, vol. iii. (1862), p. 125.

[3] *Quart. J. Microsc. Science*, Jan. 1880, p. 106.

[4] *Monatsheft f. Chemie*, ix. 361. This paper gives the results of cultivating the bacteria by the most recent methods.

[5] *Compt. rend. Soc. Biol.* 1889, p. 438. Two other pigments produced by a variety of the bacillus (B. pyocyanicus, β) are here described ; aromatic bodies are produced as well.

CHAPTER XIX

RESPIRATION

In the foregoing chapters upon the blood, lymph, and similar fluids, little or no reference has been made to their gaseous constituents. This omission we now proceed to supply, judging it to be more convenient to deal with the blood gases in connection with the function of respiration.

The respiratory organs consist in air-breathing animals of the lungs, in aquatic animals of the gills.[1] The respiratory system also includes the passages by which the air or the water respectively is carried to the lungs or gills, and the muscular apparatus by means of which the respiratory movements are executed, and these in turn are controlled by a nervous mechanism.

The lungs consist essentially of numerous little hollow sacs, in the walls of which is a close plexus of capillary blood vessels. These air cells, or alveoli, communicate with the external air by means of the trachea, bronchi, and bronchial tubes. Inspiration is due to a muscular effort that enlarges the thorax, the closed cavity in which the lungs are situated; owing to the atmospheric pressure the lungs become distended; the atmospheric air, however, does not actually penetrate beyond the larger bronchial tubes; the gases which get into the smaller tubes and air cells do so very largely by the process of diffusion. Expiration is ordinarily brought about by the elastic rebound of the lungs and chest walls, and is only a muscular effort when forced; but even the most vigorous expiratory effort is unable to expel the alveolar air. The alveolar air and the blood in the pulmonary capillaries are separated by the thin capillary wall, and an equally thin epithelium that lines the alveolus. The blood which is venous in the capillaries parts with its excess of carbonic acid and watery vapour to the alveolar air; this by the process of diffusion, aided by the expiratory efforts, passes into the atmosphere. The blood at the same time receives from the alveolar air a supply of oxygen which renders it arterial.

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[1] Insects possess a number of tracheæ or air-tubes kept open by a spiral of chitin; these penetrate to all parts of the body and supply the requisite oxygen. Pulmonary sacs are found in certain groups of invertebrates (*e.g.* Arachnida); gills are present in most aquatic invertebrates.

In fish the supply of oxygen is derived from the air dissolved in water. The capillaries of the gills come into close contact with the water, which not only parts with some of its dissolved oxygen, but receives from the venous blood the products of combustion, of which the most important is carbonic acid.

In the interchange of gases that occurs in the essential organ of respiration, diffusion plays a certain part, but this is aided by chemical processes; for instance, the union of oxygen with hæmoglobin.

The intake of oxygen and the output of carbonic acid are, however, only parts of the function known as respiration. The intake of oxygen is the commencement, and the output of carbonic acid is the end, of the series of changes. The intermediate steps take place all over the body, and constitute what is known as tissue-respiration. The compound oxyhæmoglobin is only a loose one, and in the tissues it parts with its oxygen supplying them with this element. This oxygen does not necessarily undergo immediate union with carbon to form carbonic acid, and hydrogen to form water, but in many cases, as in muscle, is held in reserve by the tissue itself. Ultimately, however, the two oxides just mentioned are formed; they are the chief products of combustion. There are other products, such as the imperfectly oxidised substances (urea, uric acid, &c.) that pass into the urine. These products of combustion pass into the venous blood, and the gaseous products, carbonic acid, and a portion of the water in the form of vapour find an outlet by the lungs.

Such is a brief account of the various steps that constitute respiration. These we have now to discuss one by one.

THE GASES OF RESPIRATION

Methods of Investigation

The methods, by means of which the changes in the air brought about by respiration can be investigated, are two in number:—

1. An animal is placed in a closed chamber; the carbonic acid formed is continually removed, and the necessary oxygen supplied in measured quantities. This is the principle of the method of Regnault and Reiset.

2. The animal is placed in a chamber through which atmospheric air is passed, and the change in the composition in the air after passing through the chamber is examined. This is the principle of the method of Scharling,[1] and later of Pettenkofer.

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[1] *Ann. Chem. Pharm.* xlv. 214.

The method of Regnault and Reiset.[1]—The apparatus consists of a bell jar, R, in which is placed the animal to be experimented on. This is placed in a cylinder, *gg* (provided with a thermometer *t*), by which the temperature can be regulated, or which can be employed for calorimetric experiments. A tube, *b*, leads into the bell jar R, and through it passes a known volume of oxygen; to absorb any trace of carbonic acid a vessel containing potash is placed on the course of the tube. Two tubes, *d* and *e*, lead from R, and are united by caoutchouc tubes with the potash bulbs, which can be raised or depressed alternately by the beam W. In this way they aspirate the air from R, and the increase of weight in the potash bulbs indicates the amount of carbonic acid expired. The manometer *f* indicates the difference of pressure, if any, outside and inside R.

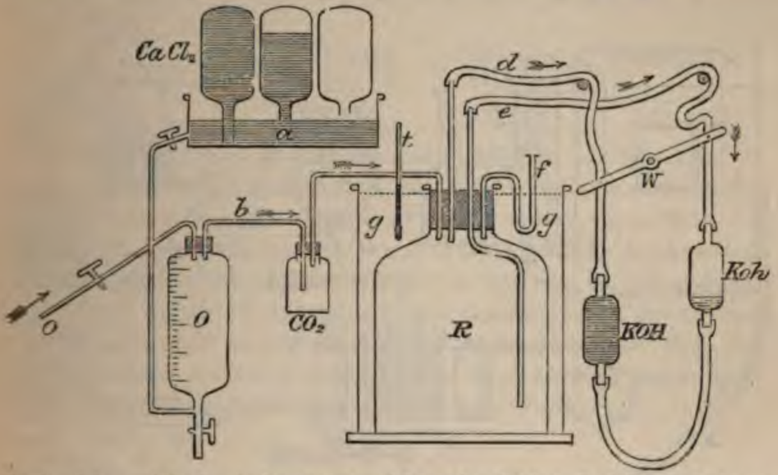

FIG. 64.—Scheme of the Respiration Apparatus of Regnault and Reiset. R, globe for animal; *g*, *g*, outer casting for R, provided with a thermometer, *t*; *d* and *e*, exit tubes to movable potash bulbs, KOH and K*oh*; O, ingoing oxygen; CO_2, vessel to absorb any carbonic acid; $CaCl_2$, apparatus for estimating the amount of O supplied; *f*, manometer.

Special modifications of respiration apparatuses on this same principle have been introduced by Pflüger, Zuntz, Finkler, Oertmann, Schulz, and Stroganow.[2]

The method of Pettenkofer.[3]—The apparatus used consists of a chamber Z with metallic walls, provided with a door and window. At *a* is an opening for the admission of air, while a large double-suction pump (PP_1) continually renews the air through the chamber. The air passes into a vessel *b* filled with pumice stone saturated with sulphuric acid, in which it is dried; it also passes through a gas meter C, which measures its volume. The air is emptied outwards by means of PP_1; from the chief exit tube *x*, provided with a manometer *g*, a laterally placed tube *n* passes, conducting a small secondary stream which is chemically investigated. This current passes through the suction apparatus MM_1 (driven like PP_1 by a steam engine), but on its way goes through the bulb K (filled with sulphuric acid); the increase in weight of this bulb gives the amount of watery vapour. After MM_1 it passes through the tube R filled with baryta water which takes up the carbonic acid. The quantity of air which passes through the secondary current *n* is lastly measured by the gas meter *u*. An accessory stream N enables the investigator to examine the air before it enters the chamber, and is arranged in the same way as *n*. The increase of water and carbonic acid in *n*, as compared with N, furnishes the data from which calculations are subsequently made.

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[1] *Ann. de chim. et de phys.* sér. 3. vol. xxvi. p. 299 (1849); vol. lxix. (1863).

[2] *Pflüger's Archiv*, iv. 83; xii. 25; xiv. 38, 78.

[3] *Sitzungsb. d. bayer. Akad. d. Wiss.* 1862, vol. ii. pp. 56, 88; 1863, vol. i. p. 152. *Ann. Chem. Pharm.* suppl. vol. ii. p. 1, 1862.

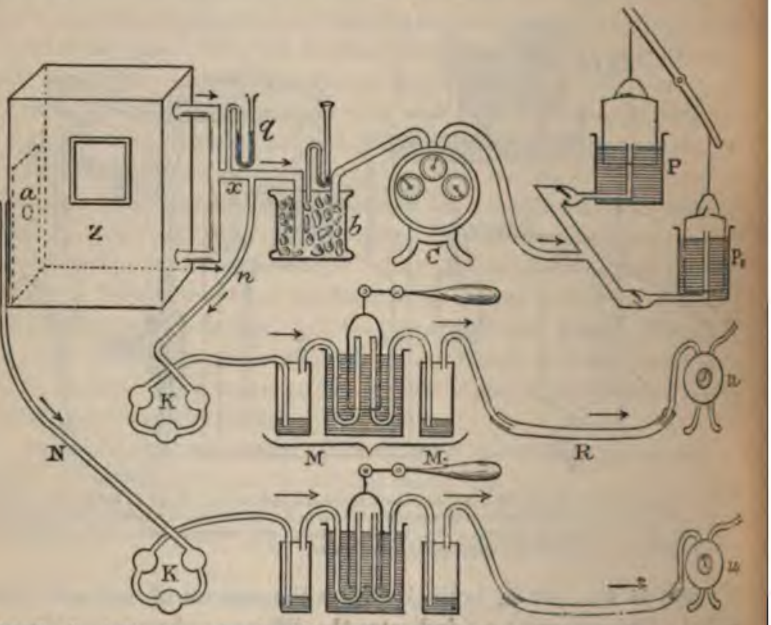

FIG. 65.—Respiration Apparatus of v. Pettenkofer. Z, chamber for person experimented on; *x*, exit tube with manometer, *q*; *b*, vessel with sulphuric acid; C, gas-meter; PP_1, pump; *n*, secondary current, with *k*, bulb; MM_1, suction apparatus; *u*, gas-meter; N, stream for investigating air before it enters Z.

The chief investigations made by this method are those of Pettenkofer and Voit[1] on healthy and diseased human beings; the food, excretions, amount of work, and other varying conditions being taken into account. The great advantage of the method is the very short stay in the chamber necessary for an experiment. W. Henneberg[1] made numerous experiments on the lower animals by the same method. Haldane and Pembrey[2] have recently described simpler methods by which moisture and carbonic acid can be estimated. They use soda-lime to absorb the carbonic acid.

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[1] *Sitzungsb. d. bayer. Akad. d. Wiss.* Nov. 10, 1866; Feb. 9, 1867.

Atmospheric Air

The density of the air varies with temperature and barometric pressure, but at 0° C. and a pressure 760 mm. of mercury dry air consists of :—

	Oxygen	Nitrogen	Carbonic acid
By weight per cent.	23·015	76·985	
By volume "	20·96	79·02	0·03–0·034

In round numbers the air contains one fifth of its volume of oxygen, and a mere trace of carbonic acid. It contains a still smaller amount of ammonia. The amount of watery vapour varies with the temperature, increasing as the temperature rises. It is, however, necessary to distinguish between the *absolute moisture*, i.e. the quantity of vapour per volume of air, and the *relative moisture*, i.e. the amount of vapour per volume of air with respect to its temperature. Thus, the air in the summer contains absolutely three times as much vapour as in winter, but relatively it is drier than the air in winter.

The Expired Air

The average percentage composition of expired air is by volume

Oxygen	Nitrogen	Carbonic acid
16·033	79·03	4·38 (from 3·3 to 5·5)

The expired air contains nearly a hundred times more carbonic acid than the inspired air, and 4·827 vols. per cent. less oxygen. More oxygen is taken into the body than carbonic acid is given off ; there is thus a slight diminution ($\frac{1}{40}-\frac{1}{50}$) in the volume of the expired as compared with the inspired air ; but this diminution is far more than compensated by the warming which the inspired air undergoes in the respiratory passages, so that the actual volume of the expired air is rather greater than that of the inspired air. The relation $\frac{\text{CO}_2 \text{ given off}}{\text{oxygen absorbed}}=\frac{4·38}{4·827}=0·907$ is called the respiratory quotient. This is, however, very variable

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[1] *Neue Beitrage zur Begr. einer rationellen Fütterung*, Heft i. Göttingen 1870–72

[2] *Philosoph. Mag.* April, 1890

The loss is due to the fact that all the oxygen taken in does not combine with carbon to form carbonic acid, but some is used up in the formation of water, urea, &c.

The temperature of the expired air is very nearly that of the body (36·3° C.), and remains constant even though the temperature of the atmosphere varies.

The nitrogen of the expired air is practically unchanged. Regnault and Reiset state that a small quantity of nitrogen is added to the expired air[1] (except in starving animals where a small quantity is absorbed) and also that traces of ammonia, hydrogen, and marsh gas (CH_4) and other organic impurities are present. It is probable that these gases diffuse from the intestines.

The expired air lastly is saturated with watery vapour. Hence, as the vapour in the atmosphere varies, the lungs must give off different quantities of water from the body.

Diffusion of Gases within the lungs

If we compare the air at the entrance of the respiratory system (the mouth and nose) with that in the air cells, which can be obtained by catheterisation, it will be found that whereas the former differs but little from the atmospheric air in composition, the latter contains less oxygen and more carbonic acid. Between these two extremes, that is, in intermediate portions of the respiratory tubes, there is an intermediate condition in the proportion of the two gases. The oxygen must, therefore, tend to diffuse inwards from the atmosphere, and the carbonic acid outwards from the air vesicles. In hibernating animals the exchange of gases takes place solely in this way, but ordinarily the respiratory movements aid diffusion, atmospheric air being introduced into the larger air-passages, and draughts created so that the movement of the gases is accelerated.

Quantity of Gases respired

Residual air is that which remains in the lungs after the most complete expiration. It is equal to 100–130 cubic inches (1200–1600 c.c.).

Reserve or supplemental air is the volume of air which can be forcibly expelled, after a normal expiratory effort. It is equal to about 100 cubic inches (1200–1800 c.c.).

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[1] This remarkable conclusion is according to Pettenkofer and Voit (*Zeit. Biol.* i. Heft i.; xvi. p. 508) due to experimental errors. Seegen and Nowak (*Wiener Akad. Sitzungsber.* lxxi. Abth. iii. 1875; *Pflüger's Biol.* xxv. 383), however, using a Regnault's apparatus, confirmed Regnault and Reiset's observations on this point. *See also* Leo, *Pflüger's Arch.* xxvi. 218, and Reiset, *Compt. rend.* xcvi. 549.

Tidal air is that which is taken in and given out with each quiet respiration. It is equal to 20 cubic inches (500 c.c.).

Complemental air is that which can be forcibly inspired over and above that taken in at a normal respiration. It is equal to 100–130 cubic inches (1500 c.c.).

Vital capacity is the volume of air which can be forcibly expelled after the deepest possible inspiration. It = reserve air + tidal air + complemental air = 230 cubic inches (3800 c.c.). These numbers were obtained by Hutchinson by means of the instrument he invented and called the spirometer, a special form of gasometer adapted for the purpose.

The above numbers are averages. Varying conditions which modify the vital capacity are *height* (one inch in height increasing it by eight cubic inches); *weight* (when the weight exceeds the normal by seven per cent. each kilogramme of increase diminishes the vital capacity by 2–3 cubic inches); *age* (after 35 it gradually diminishes); *sex* (in a man and woman of the same height the ratio is 10 : 7); *position* and *diseases*.

The frequency of quiet respiration varies with age; the following average numbers are given by Quetelet[1]: in newborn children, 44; at the age of five, 26; at the age of fifteen to twenty, 20; twenty to twenty-five, 18; twenty-five to thirty, 16; thirty to fifty, 18 respirations per minute. Hutchinson gives 16–24 respirations per minute as the average of 2000 observations.

	cubic metres		cubic feet
The total air respired in the twenty-four hours	= 11	=	330
	litres		
,, ,, per hour	458	=	13·7

Vierordt gives the following figures :—

	grammes	
Oxygen taken in in the twenty-four hours	=744	=516,500 c.c.
Carbonic acid given out ,,	=900	=455,500 c.c.

The excess of oxygen absorbed over carbonic acid expired is thus 61,000 cubic centimetres in the day; most of this combines with hydrogen to form water, and a small quantity is contained in urea, uric acid, &c. Dumas gives the total quantity of carbon exhaled in carbonic acid as 8½ oz. in the twenty-four hours; E. Smith as 7–11 oz.

The aqueous vapour averages between 350 and 500 grammes. But this is very variable, the chief factor in the variation being the quantity already present in the inspired air which is dependent on climate, temperature, &c.

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[1] *See* Ranke, *Grundriss d. Physiol. d. Menschen*, p. 353, Leipzig, 1868.

The effects of varying circumstances on Respiration

Many circumstances affect the respiratory exchanges, particularly with reference to the carbonic acid : such as state of rest or activity, food, day and night, sleep, sex, age, mode of respiration, season of year, alterations of atmospheric pressure, &c.

Age.—Until the body is fully developed the carbonic acid given off increases with age ; as the bodily energies decay it diminishes. Hence the oxygen absorbed is relatively greater than the carbonic acid given off. The absolute amount of carbonic acid given off is less in children than adults, but in relation to body weight a child gives off twice as much as an adult. The following table is taken from Landois and Stirling's Physiology :—

Age	In 24 Hours	
	Amount of Carbonic Acid Excreted	Amount of Oxygen Absorbed
8 years	443 grammes of CO_2 = 121 gr. of C.	375 grammes
15 "	766 " " = 209 "	652 "
16 "	950 " " = 259 "	809 "
18–20 "	1003 " " = 274 "	854 "
20–24 "	1074 " " = 293 "	914 "
40–60 "	889 " " = 242 "	757 "
60–80 "	810 " " = 221 "	689 "

Sex.—After the eighth year males give off about one third more CO_2 than females (Andral and Gavarret). At puberty the difference may rise to one half. Pregnancy increases the output of carbonic acid.

Temperament.—Energetic muscular people absorb more oxygen and excrete more carbonic acid than less active persons of the same weight.

Day and Night.—C. Schmidt[1] was the first who found that the output of carbonic acid during the night is diminished. Pettenkofer and Voit arrived at the same result. The cause is that during sleep the respiratory, like all the other functions of the body, is less active than in waking hours. The influence of light in increasing the output of carbonic acid has been investigated by numerou experimenters,[2] and appears to be explicable on the supposition that muscular movements are more active in the light than in the dark, or during sleep.

Hibernation.—Respiration is enormously diminished, and the exchange of gases is carried out by diffusion and the cardio-pneumatic movements. The carbonic acid given off falls to $\frac{1}{75}$, the oxygen taken in to $\frac{1}{41}$ of what they are during active life (Valentin). The body weight may increase through the relative excess of oxygen ; and according to Regnault and Reiset a small quantity of atmospheric nitrogen is also absorbed.

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[1] Bidder and Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, Milan and Leipzig, 1852, p. 367.

[2] Moleschott (*Chem. Centralbl.* 1872, No. 49), Pott (*Habilitationsschrift*, Jena, 1875), Pflüger and v. Platen (*Pflüger's Arch.* xi. 272). *See* also p. 211.

Surrounding temperature.—The temperature of cold-blooded animals rises and falls with that of the atmosphere, and the amount of chemical, including respiratory, activity varies similarly. Moleschott states that a frog at 39° C. excretes three times as much carbonic acid as when the temperature was 6° C.

In warm-blooded animals, however, the body temperature remains constant amid the variations of that of the surrounding air. As the atmospheric temperature diminishes, the processes of oxidation within the body are necessarily increased; this is brought about by a reflex nervous mechanism; the increase of chemical changes produces an increased amount of heat, so as to keep the body temperature up to the normal level. The reverse processes obtain when the atmospheric temperature increases (Lavoisier,[1] Sanders-Eyn,[2] Vierordt,[3] Colasanti,[4] Theodor,[5] Voit,[6] Page[7]).

The following is the mean result of 21 experiments by Colasanti on guinea-pigs:—

For 1 kilogram Body Weight per Hour	At the Mean Temperature of	
	3·6° C.	26·2°
Oxygen absorbed	1856·5 c.c.	1118·5 c.c.
Carbonic acid excreted	1554·8 „	1057·4 „
Respiratory quotient $\frac{CO_2 \text{ excreted}}{O \text{ absorbed}}$ . .	0·83 „	0·94 „

Page experimenting on dogs found that there is a temperature of the surrounding medium at which the carbonic acid is at a minimum (about 25° C.); below this temperature the quantity of carbonic acid discharged increases as the temperature falls; above this the discharge also increases, and at abnormally high temperatures (40°–42°) the increase may be very rapid.

Fever.—In fever the body temperature is raised, and this brings with it, as in cold-blooded animals, an increase of chemical activity. In Page's experiments with the high temperatures just mentioned, the body temperature of the animal, as well as that of the air, was raised.

The following were the results obtained by Pflüger and Colasanti in guinea-pigs:—

Temperature of Animal in Rectum	Oxygen Absorbed	Carbonic Acid Expired	Resp. Quotient
37·1° C.	948·17	872·06	0·92
38·5°	1137·3	949·5	0·83
39·7°	1242·6	1201·59	0·96

Liebermeister[8] has made similar observations in cases of typhoid and intermittent fever in human beings. It will be sufficient to quote one example; this shows how, in the quickly succeeding phases of an attack of ague, the output of carbonic acid and the production of heat run parallel.

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[1] *Oeuvres*, vol. ii. p. 688.

[2] *Ber. d. sächs. Akad. d. Wiss.* 1867, p. 58.

[3] *Physiol. des Athmens*, Karlsruhe, 1845.

[4] *Pflüger's Archiv* xiv. 92, 471.

[5] *Zeit. Biol.* xiv. 51.

[6] *Ibid.* p. 57.

[7] *Journ. Physiol.* ii. 228.

[8] *Handbuch der Path. u. Therap. d. Fiebers*, Leipzig, 1875, pp. 327–340.

	Output of CO_2	Heat-Production
In the first half-hour	13·85	44
„ second „	19·07	61
„ third „	34·49	1 10
„ fourth „	19·50	62
„ fifth „	17·99	58
„ sixth „	17·15	55

Leyden and Frankel[1] have made similar observations.

Other pathological conditions have not been so fully worked out as fever; it may, however, be stated that diseases of the lungs which diminish their capacity for respiratory purposes necessarily lead to a diminished respiratory exchange. In such cases, as well as in cases of obstructive disease of the respiratory passages the inspiration of compressed air would be of great benefit. In the normal condition the blood takes from compressed air very little more oxygen than at the ordinary barometric pressure; this is because the gas in the blood is chiefly in a state of chemical union with hæmoglobin, not in a condition of simple solution (*see* further under Gases of the Blood). But when the volume of the lungs is diminished the hæmoglobin is unable to get all the oxygen necessary to form a due quantity of oxyhæmoglobin; but air occupying the same volume, but containing a greater supply of oxygen, i.e. compressed air, would obviously correct this.

Food.—In inanition the output of carbonic acid and the intake of oxygen both diminish, especially the former, and thus the respiratory quotient falls (0·73) (Bidder and Schmidt, Pettenkofer and Voit, Regnault and Reiset); a small quantity of atmospheric nitrogen is absorbed (Regnault and Reiset).

An increase of respiratory activity occurs after meals, especially about an hour after the chief meal (Vierordt). Substances rich in carbon (starches and fats) cause an increased excretion of carbonic acid. A purely carbohydrate diet is only compatible with life for a short time; but during this time the respiratory quotient rises to unity, or almost so; this is because the hydrogen of the food is already fully oxidised, hence the oxygen inspired has virtually only carbon to combine with (Regnault and Reiset). Substances which are oxidisable, like sodic lactate, glycerine, &c., when injected into the blood stream cause an increase of the oxygen taken in and the carbonic acid given out (Ludwig and Scheremetjewsky). Alcoholic drinks (especially brandy, whisky, and gin—E. Smith), tea and ethereal oils diminish the output of carbonic acid (Prout, Vierordt).

Muscular Activity.—Muscular contraction causes a great increase in the output of carbonic acid, and in the intake of oxygen, but especially in the former, so that the respiratory quotient, $\frac{CO_2}{O_2}$, rises. This was originally pointed out by Lavoisier, but has been more especially worked out by the researches of Ludwig and Sczelkow.[2] If the venous blood leaving a muscle during rest be examined and compared with that which leaves the muscle during activity, it will be found that in the latter case the carbonic acid in the blood will be more increased and the oxygen more diminished than in the former. A few examples from these experiments are given in the following tables:—

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[1] *Centr. med. Wiss.* 1875, No. 39.

[2] *Wien. Akad. Sitzungsber.* xlv. (1862)

	Arterial Blood			Gases of Venous Blood from Resting Muscle			Gases of Venous Blood from Active Muscle		
	O_2	N_2	CO_2	O_2	N_2	CO_2	O_2	N_2	CO_2
I.	16·06	1·2	29·26	3·74	1·2	38·42	1·51	1·2	40·52
II.	17·33	1·6	24·54	7·50	1·4	31·6	1·26	0·92	34·88

If the expired air be analysed instead of the blood gases, analogous results are obtained. The following example is taken also from Sczelkow's work. Rabbits were the animals employed:—

During	C.C. in One Minute		Respiratory Quotient
	CO_2 Expired	O_2 Absorbed	
I. Repose	4·97	12·29	0·404
Tetanus	13·69	12·11	1·13
II. Repose	7·85	12·76	0·615
Tetanus	17·62	19·02	0·927
III. Repose	6·99	17·47	0·400
Tetanus	19·61	30·35	0·646

The following example is from experiments on human beings:—

Discharge of	On Deficient Diet		On Moderate Diet	
	During Repose	During Work	During Repose	During Work
CO_2	695 grms. per diem	1187	930	1134
H_2O	814 „ „	1177	967	1412
Urea	26·3 „ „	26	37·2	37·3
Intake of O_2	743 „ „	1042	867	1006

Experiments on man by Vierordt, Speck, and Pettenkofer and Voit, and on horses by F. Smith,[1] all gave the same result. In curare poisoning, where the muscles are inactive, there is much diminished respiratory exchange of gases (Zuntz).[2]

The increase of the gaseous exchanges during forcible respiration is partly explicable by the increase of muscular work.

Increased work of the involuntary muscles also produces the same result. The stomach and intestinal tract have been investigated in this direction (v. Mering and Zuntz,[3] A. Loewy[4]). Loewy's experiments were carried out both on rabbits and men; when the activity of the intestinal tract is increased by saline purges, there is a rise in the respiratory gaseous exchanges. No doubt this increased metabolism is due to the activity both of the muscular tissue and the glands of the intestine, but probably the former is the more important factor concerned. This subject is of practical interest, as, therapeutically, the cures of Carlsbad, Marienbad, &c., consist in increasing the activity of the alimentary canal by means of saline purgatives.

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[1] *Journ. of Physiol.* xi. 65. Zuntz and Lehmann (*Zeitsch. f. wiss. handwirthsch.* 1889 *Journ. of Physiol.* xi. 396) have also made similar experiments with horses.

[2] *Pflüger's Archiv*, xii. 522.

[3] *Ibid.* xv. 634; xxxii. 173.

[4] *Ibid.* xliii. 515.

Other forms of activity.—Not only does muscular work increase the amount of gaseous interchange, but all forms of protoplasmic activity act similarly; chemical decompositions are most rapid and extensive when an organ is active. Among forms of activity, secretion is the most important after muscular contraction.

Number and Depth of Respirations.—The most marked effect of increasing the respiratory movements is not to influence the amount of carbonic acid formed in the body, but to accelerate the removal of that which has been already formed.

An increase in the number of respirations (the depth remaining the same), or an increase in their depth (the number remaining the same), causes an increase in the amount of carbonic acid given off, though with reference to the total amount of gases exchanged it is relatively diminished. This may be illustrated by the following table from Vierordt:—

No. of Respirations per Minute	Volume of Air	Amount of CO_2 = CO_2 per cent.	Depth of Respirations	Amount of CO_2 = CO_2 per cent.
12	6,000 c.c.	258 c.c. = 4·3 per cent.	500	21 c.c. = 4·3 per cent.
24	12,000 ,,	420 ,, = 3·5 ,,	1000	36 ,, = 3·6 ,,
48	24,000 ,,	744 ,, = 3·1 ,,	1500	51 ,, = 3·4 ,,
96	48,000 ,,	1392 ,, = 2·9 ,,	2000	64 ,, = 3·2 ,,
			3000	72 ,, = 2·4 ,,

Corroborative results have been obtained by Voit and Lossen,[1] Speck,[2] Berg,[3] and Becher.[4]

Deficiency of Air or of Oxygen. Dyspnœa. Asphyxia—When a due supply of air is not obtained the oxygen in the arterial blood sinks below the normal, the blood pressure rises, and the respiratory movements become deeper (dyspnœa or hyperpnœa); these movements increase until they pass to other muscles, and so a condition of general convulsions sets in; this is followed by exhaustion and death; the train of symptoms constituting what is known as asphyxia.

An increased supply of Air or Oxygen. Apnœa.—After several inspiratory efforts of great force it is easy to hold the breath for a longer time than usual. If air be rapidly pumped into the lungs of one of the lower animals there is no effort made to breathe for the space of some seconds or even minutes. The usual explanation given of these phenomena is as follows: the respiratory centre in the medulla is largely influenced by the quality of the blood sent to it. In ordinary respiration, the normal blood not being fully oxygenated stimulates it to send out impulses which result in the normal respiratory efforts: too great an amount of carbonic acid in the blood excites it to increased activity (dyspnœa); too large a supply of oxygen inhibits its activity altogether (apnœa). This view of the cause of the respiratory movements is, however, not universally accepted. Thus Hoppe-Seyler[5] states that normal arterial blood contains no such reducing substances as have been considered stimulants of the respiratory centre. He is inclined to believe that the excitation to respiratory activity is to be found in the changes that occur in the lungs; these stimulate the terminations of the sensory nerves in the lungs, and by means of reflex action the muscular movements of the respiratory muscles are brought about. Max Marckwald[1] also adduces many weighty arguments against the generally received theory of respiration. Dyspnœa seems to be undoubtedly caused by excess of carbonic acid in the blood, whether this affects the nerve centre or the nerve terminations. But apnœa, according to Hoppe-Seyler,[2] is not caused by excess of oxygen in the blood, as normal arterial blood is already completely or almost completely saturated with oxygen; from the study of his own experiments and those of other investigators,[3] he concludes that it is simply due to fatigue of the respiratory apparatus.

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[1] *Zeit. Biol.* ii. 244.

[2] *Schriften d. Gesellsch. z. Forder. d. Ges. Naturwiss.* Marburg, x. 3.

[3] *Deutsch. Arch. klin. Med.* vi. 291.

[4] *Die Kohlensäurespannung im Blut*, Zürich, 1855.

[5] *Physiol. Chem.* p. 544.

Poisonous Gases.—Excess of carbonic acid produces feelings of discomfort (headache, &c.); if the excess is very great there is laboured breathing, and ultimately a state of narcosis without convulsions, in which the animal dies. Carbonic oxide is even more deleterious; it combines with the hæmoglobin, and so prevents the blood, and thus the tissues, from being properly oxygenated (*see* p. 281).

Sulphuretted hydrogen acting as a reducing agent produces similar effects. Some gases, like chlorine, ammonia, nitrous acid, &c., are irrespirable, producing spasm of the glottis. Nitrous oxide causes narcosis, and is largely used as an anæsthetic.

Ozone, instead of making the blood more arterial, causes it to assume venous characters in all the vessels; this is perhaps explained by its greater density, interfering with the due excretion of carbonic acid from the blood; it also causes local irritation of the respiratory passages; it slows both heart and respiration (Dewar and McKendrick,[4] J. Barlow,[5] Filipow[6]). Hydrogen and marsh gas, if mixed with a sufficient quantity of oxygen, have no effect on respiration.

The poisonous effects of respired air.—Dr. B. W. Richardson[7] has made experiments on animals in order to investigate the effect of air that has been breathed previously by other animals. He finds that after air or oxygen has been once used it is very poisonous, even though all carbonic acid, ammonia, and all appreciable impurities have been removed. He therefore infers that something is removed from the oxygen by the process of respiration; and that this 'devitalised oxygen' can be revitalised by electrical brush discharges from the positive pole of a frictional machine.

As long as oxygen is regarded as an element, it is impossible to accept this explanation. A much more probable explanation is that some impurity is added to the oxygen during the process of respiration, and that this impurity is the poison. What, then, is this impurity? Jackson[8] considers it may be carbonic monoxide. What seems to be certain is that it is not carbonic dioxide; a large admixture of pure carbonic acid in the air will not produce the symptoms of poisoning. Men can breathe for two to three hours without marked discomfort air which contains as much as 20 per cent. of carbonic acid. Brown-Séquard and d'Arsonval[9] speak of the poison vaguely as a pulmonary poison, and consider it may be alkaloidal, and that it passes into the expired air from the lungs; this poison, whatever it is, can be removed by passing the air containing it through tubes containing beads moistened with sulphuric acid.

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[1] *Innervation of Respiration*, translated by T. A. Haig: Blackie and Son, 1888; *Zeit. Biol.* xxvi. 259. *See* also H. Head, *Journ. of Physiol.* x. 1.

[2] *Physiol. Chem.* p. 519.

[3] Rosenthal, *Arch. d. Anat. u. Physiol.* 1864, p. 456; 1865, p. 191. Pflüger, *Pflüger's Archiv*, i. 90. A. Ewald, *Ibid.* vii. 575; also 'Ueber die Apnoë,' *Diss.* Bonn, 1873.

[4] *Proc. Roy. Soc.* 1873–4.

[5] *Journ. Anat.* Oct. 1879.

[6] *Pflüger's Archiv*, xxxiv. 335.

[7] *Brit. Med. Journal*, vol. ii. 1860. *Chem. News*, lv. 253.

[8] *Proc. Physiol. Soc.* 1887, p. 31.

[9] *Comptes rend.* 1887, 1888, 1889.

Although the mere presence of 1 per cent. of *pure* carbonic acid in the air has little or no effect, an atmosphere in which the carbonic acid has been raised to this proportion *by respiration* is highly detrimental; indeed, air rendered so impure by respiration as to contain even 0·08 per cent. of carbonic acid is very unwholesome. In an hour a man will add about 1 per cent. of the gas to about 70 cubic feet of air; and if the proportion is kept down to 0·1 per cent., at least 700 cubic feet should be supplied to him every hour, or about 16,800 cubic feet in the 24 hours.

Changes in atmospheric pressure.[1]—Gradual diminution of pressure produce symptoms of asphyxia; convulsions, however, are not invariable. A sudden and great diminution of pressure may produce death by the liberation of nitrogen within the blood vessels, and a consequent mechanical interference with the circulation. Increase of pressure up to that of several atmospheres produces symptoms of narcotic poisoning; at a pressure of 20 atmospheres the animals die of asphyxia, as when oxygen is deficient. The oxidations in the body are at this pressure diminished. Plants, bacteria, &c., are similarly killed by too great pressure of oxygen; and at a high pressure of oxygen even phosphorus will not burn.

It is, however, only very great extremes of pressure that affect animals injuriously. As is explained more fully in connection with the subject of the blood gases, very considerable variations of pressure may take place, especially if gradual, and without any resulting inconvenience to the animal. A mere excess of oxygen in the air breathed has no appreciable influence either on the amount of oxygen taken up or carbonic acid given out by the animal.[2]

Marcet[3] states that less air (reduced to 0° C. and 760 m.m.) is taken into the lungs for the formation and emission of a given weight of carbonic acid under lower than under higher atmospheric pressures.

THE GASES OF THE BLOOD

H. Davy[4] was the first to observe that oxygen was evolved on heating the blood. Magnus[5] made more accurate observations. He found that oxygen could be obtained either by passing a stream of hydrogen or carbonic acid through the blood, or by placing blood in the vacuum of an air pump, and that the quantity of oxygen obtained from arterial was greater than that from venous blood. Later Bunsen,[6] Lothar Meyer,[7] and later still after the invention of the mercurial air pump Hoppe-Seyler, Setschenow and Ludwig, Helmholtz, and Pflüger worked at the subject.

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[1] Paul Bert, *Recherches exp. sur la pression barométrique*, 1874.

[2] Some recent experiments on this subject by Saint-Martin will be found in *Compt. rend.* xcviii. 241.

[3] *Phil. Trans.* vol. clxxxi. (1890), p. 1.

[4] *Gilbert's Ann.* xii. 593.

[5] *Poggendorff's Ann.* xl. 583 (1838); lxvi. 177.

[6] Bunsen, *Gasometrische Methoden*, Braunschweig, 1857.

[7] L. Meyer, *Die Gase des Blutes, Diss.* Göttingen, 1857. *Zeit. rat. Med.* viii. 25

The general principles underlying the construction and use of the mercurial air pump have been described in an earlier chapter (*see* p. 30). It will be here unnecessary to repeat the principles on which the analysis of the gases is performed (*see* Chap. IV), and we can pass on now to the results that have been obtained.

The following table[1] gives some numbers illustrative of the results obtained by different observers :—

Observer	Kind of Blood	Percentages per Volume[2]		
		Oxygen	Carbonic Acid	Nitrogen
L. Meyer[3]	Dog (from carotid) .	12–18	26–34	3–5
	Calf (defibrinated) .	11–5	20	4·4
Setschenow[4]	Dog (from carotid) .	19–21	40–43	1·6
Schöffer[5]	„ (from artery) .	14–22	34–43	1·6–2·5
	„ (from vein) . .	5–15	38–47	1·3–1·6
Sczelkow[6]	„ (from artery) .	15–22	32–37	1·2–2·1
	„ (from vein) . .	1·6–10	41–52	1·2–1·8
Nawrocki[7]	„ (from carotid) .	10–20	27–45	1·2–2·5
H. Hirschmann[8]	(from carotid, 3 experiments)	27, 16, 13	24, 40, 37	3, 2, 2
	(from femoral in the same dogs)	25, 16, 15	19, 42, 36	5, 2, 2

Jolyet and Regnard[9] have made observations on the blood gases of several aquatic animals, crustacea and fishes (*see* also p. 396).

Pflüger,[10] whose name is associated with the best known of the many mercurial pumps, from a large number of observations on dogs found in the arterial blood in the mean 58·3 volumes of the mixed gases per 100 vols. of blood ; this was composed of 22·2 vols. oxygen (maximum 25·4) ; 34·3 vols. carbonic acid ; and 1·8 vols. nitrogen.

Pflüger[11] made, like Hirschmann, comparative observations on the gases of blood obtained from different arteries (carotid and femoral), and his results were practically the same ; viz. the gases are in the two cases approximately equal, or there may be a slight loss of oxygen in the more distant artery.

During the first few minutes after blood is shed, a certain quantity of the oxygen disappears ; in all probability this is stored or used up by the still living corpuscles. Stroganow[1] gives the loss of oxygen that occurs in this way as varying from 1·03 to 1·6 per cent. Schützenberger,[2] by using indigo-white to remove the oxygen from hæmoglobin, obtained a result higher by 4–5 c.c. per 100 c.c. of blood than by the method of extracting the gas with the mercurial air pump. This difference increases with the length of time employed in the extraction of gas by the pump. Lambling[3] considers that this is in part due to the formation of a small amount of methæmoglobin. Methæmoglobin yields its oxygen to reducing agents like indigo-white, but not to the vacuum of an air pump. Hoppe-Seyler[4] states that the reduction by means of indigo-white is not trustworthy, since it is so powerful. Not only is oxyhæmoglobin reduced to hæmoglobin, but the reaction goes further, so as to form hæmochromogen. Lambling, however, from numerous experiments considers that Hoppe-Seyler is at fault here; hæmoglobin is formed, and the reaction always stops short there. The question cannot, however, be regarded as settled.

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[1] Shortened from a fuller table given by Hoppe-Seyler, *Physiol. Chem.* p. 496.

[2] In all cases the volume of gas is measured at 0° C. and 760 m.m. Hg pressure.

[3] *Loc. cit.*

[4] *Wien. akad. Sitzungsb.* xxxvii. 293.

[5] *Ibid.* xli. 589.

[6] *Ibid.* xlv. 171.

[7] Heidenhain, *Studien d. physiol. Inst. zu Breslau*, Heft ii. 1863, p. 162.

[8] *Archiv f. Anat. u. Physiol.* 1866, p. 502.

[9] *Virchow-Hirsch. Jahresb.* 1874, vol. i. p. 201.

[10] *Centralbl. med. Wiss.* 1867, p. 724.

[11] *Pflüger's Archiv*, i. 285.

The oxygen in the blood is not in a state of simple solution. According to Bunsen[5] the absorption-coefficient of water for oxygen is 0·041; for nitrogen 0·0203 (0° C., 760 mm. Hg). If the absorption coefficient of blood were equal to that of water, the blood would be able to absorb from the air 0·86 vols. per cent. of oxygen, and 1·608 vols. of nitrogen. The absorption coefficient of blood is a little lower than that of water, and this is smaller still at the temperature of the body (37° C.) than at 0° C., the temperature for which the above numbers are calculated. With regard to *the nitrogen*, the quantity found in the blood is explained by simple solution. But the oxygen is far in excess of what can be accounted for in this way; the oxygen is in fact nearly all present in the form of oxyhæmoglobin; in venous blood a variable quantity of oxyhæmoglobin is found mixed with hæmoglobin. Supposing that 100 c.c. of arterial blood contain 14 grammes of oxyhæmoglobin, this would account for 23·43 vols. per cent. of oxygen; this is rather more than Pflüger actually found, but all the hæmoglobin present is rarely fully saturated with oxygen.

The blood-plasma and blood-serum contain only traces of oxygen In dog's serum Pflüger[6] found 0·26 vols. per cent. of oxygen, 35·26 vols per cent. of carbonic acid, and 2·24 vols. per cent. of nitrogen.

The carbonic acid in the blood is in great part dissolved in th plasma, but it is also contained in the corpuscles. The question, in what compounds does it occur? is a difficult one to answer, since we are not able to separate them out, as we are oxyhæmoglobin, the compound in which the oxygen is contained.[1]

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[1] *Pflüger's Archiv*, xii. 48.

[2] *Bull. Soc. Chim.* 1873, p. 150.

[3] *Comptes rend. Soc. biol.* (2) v. 394, 473.

[4] *Physiol. Chem.* p. 451.

[5] Bunsen defined the coefficient of absorption of a fluid for a gas as the volume of gas (at 0° C. and 760 m.m. barometric pressure) which is taken up by one volume of fluid.

[6] *Pflüger's Archiv*, i. 73.

Al. Schmidt,[2] one of the earliest to investigate the matter, arrived at the following conclusions: (1) That the corpuscles[3] of arterial blood contain a variable amount of carbonic acid; it may amount, however, to as much as the quantity in the serum; it is relatively less in venous blood. (2) That the carbonic acid in the corpuscles can be lessened by shaking blood with oxygen; this lessening is greater than that of the carbonic acid in the serum.

Setschenow found that the carbonic acid in the corpuscles alters with the partial pressure of that gas, and indeed considers that the corpuscles are the chief source of the carbonic acid given off in the lungs.

In the serum the carbonic acid can be obtained partly by simply placing the fluid in a vacuum, but a larger yield is obtained on the addition of acid. The following are the numbers obtained by Pflüger in two experiments:—

	I	II
Carbonic acid given off *in vacuo*	44·6 vols. per cent.	35·2 vols. per cent
Additional carbonic acid given off after addition of phosphoric acid	4·9 ,, ,,	9·3 ,, ,,

In the blood itself all the carbonic acid is yielded to a vacuum without the addition of acid.

From these and other similar experiments, it appears

(1) That carbonic acid is present both in red corpuscles and serum.

(2) That serum contains the gas in a firmer union than the corpuscles.

(3) That the red corpuscles, especially if they contain oxyhæmoglobin, act in the same way as an acid, or may give rise to an acid, causing a complete expulsion of all the carbonic acid from the serum.

Zuntz[4] compared the action of a vacuum on serum with that on solutions of sodium hydrogen carbonate ($NaHCO_3$), and found that they behaved very similarly. Sertoli[5] also believes that the carbonic acid is contained in the serum as a bicarbonate, and not chiefly in union with disodium hydrogen phosphate (Na_2HPO_4), as some earli investigators stated. He shows very conclusively that the amount of phosphoric acid in the blood, if allowance be made for that contained in lecithin, is quite insufficient for the purpose. Bunge,[1] however, states that in dogs' blood it is sufficient.

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[1] Böhr (*Ludwig's Festschrift*, 1887) considers that the carbonic acid is loosely combined with the hæmoglobin itself. *See* also Jolin, *Du Bois Reymond's Arch.* 1889, p. 265.

[2] *Ber. d. sächs. Gesellsch. d. Wiss.* 1867, p. 30.

[3] It is difficult to distinguish in this relation between red and white corpuscles Setschenow, however, calculates that in 100 vols. of blood the red corpuscles contain 10 and the white 2·5, vols. of carbonic acid.

[4] *Centralbl. f. med. Wiss.* 1867, no. 532.

[5] Hoppe-Seyler's *Med Chem. Unters* Heft 3 p. 350 (1868).

One of the most remarkable phenomena in the disengagement of carbonic acid from the blood is the power of the red corpuscles to give off not only the gas they themselves contain, but also to drive off the more firmly combined carbonic acid of the serum. This is not merely due to the phosphoric acid contained in the phosphates of the stromata, nor to the proteid of the stromata. Proteid does drive out carbonic acid in a vacuum from a solution of sodium carbonate, acting in this way like an acid, but the amount driven out is very small;[2] it appears to be chiefly due to the action of oxyhæmoglobin. Preyer and subsequently Hoppe-Seyler[3] mixed solutions of pure oxyhæmoglobin and sodium carbonate together, and obtained carbonic acid from the mixture in a vacuum. As arterial blood yields its carbonic acid more easily to a vacuum than venous blood, it has been surmised that the arterial blood pigment has more of an acid character than venous blood pigment.[4]

The quantity of oxygen removable from the blood is proportional to its richness in oxyhæmoglobin. Mathieu and Urbain[5] demonstrated this by successive bleedings from the same dog; as the number of red corpuscles was thus diminished, the amount of oxygen similarly decreased; little or no change, however, was observable in the amount of carbonic acid. The amount of oxyhæmoglobin, and therefore of oxygen, is less in the blood of cold-blooded than in that of warm-blooded animals; and is among warm-blooded animals less in the blood of herbivora than in that of the dog.[6]

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[1] *Zeit. Biol.* xii. 206.

[2] Hoppe-Seyler, *Physiol. Chem.* p. 503.

[3] *Physiol. Chem.* p. 505.

[4] Hoppe-Seyler (*Zeit. physiol. Chem.* xiii. 477) draws attention to the fact that the arterial blood pigment is not the same thing as oxyhæmoglobin, and that venous blood pigment differs somewhat from hæmoglobin. He suggests the names arterin and phlebin respectively for the arterial and venous pigments as contained in the corpuscles. Arterin is probably a compound of oxyhæmoglobin with lecithin; phlebin of hæmoglobin with lecithin. The chief differences between the corpuscular pigments and the oxyhæmoglobin or hæmoglobin that can be separated from the corpuscles are: (1) the corpuscular pigments are insoluble, hæmoglobin and oxyhæmoglobin are soluble in the plasma and serum: (2) the corpuscular pigments do not crystallise readily, give off oxygen readily to a vacuum, and decompose hydrogen peroxide readily; hæmoglobin and oxyhæmoglobin behave in all these points in the opposite manner: (3) the arterial corpuscular pigment is not altered by a weak solution of ferricyanide of potassium, whereas oxyhæmoglobin is readily converted into methæmoglobin by such treatment.

[5] *Arch. de physiol. norm. et path.* iv. 14.

[6] Bunge, *Physiol. Chem.* transl. by Wooldridge, 1890, p. 256.

Changes in the Blood Gases during the circulation

The gaseous contents of the blood undergo two important changes during the course of a complete circulation. The arterial blood which leaves the left ventricle becomes venous in the tissues, losing a certain amount of its oxygen, and gaining an increased quantity of carbonic acid. The venous blood in the capillary network of the lungs loses its excess of carbonic acid, and the hæmoglobin becomes once more fully, or almost fully, oxygenated.

The nitrogen in the blood undergoes no change during the circulation if the barometric pressure remains constant; an increase of atmospheric pressure leads to the solution of a greater amount of nitrogen. An increase of pressure produces a slightly greater absorption of oxygen; that is, the quantity dissolved in the plasma is increased; the amount combined with hæmoglobin undergoes no alteration with variations of pressure unless the atmospheric pressure be so diminished (to $\frac{1}{10}$–$\frac{1}{30}$ of an atmosphere) that the point of the dissociation of oxyhæmoglobin be reached. The quantity combined with hæmoglobin is by far the most important part of the oxygen in the blood, and that this remains constant under varied barometric relations is a point of great practical interest, as it enables animals to exist even at very great altitudes where the atmospheric pressure is low, and still obtain a normal supply of oxygen. The carbonic acid in the blood undergoes practically no alterations with variations of pressure since the atmospheric air contains a mere trace of that gas. The following numbers selected from Paul Bert's[1] experiments illustrate the facts just mentioned:—

Pressure	Dog 2			Dog 5		
	O_2	CO_2	N_2	O_2	CO_2	N_2
1 atmosphere . . .	18·3	37·1	2·2	—	—	—
2 " . . .	19·1	37·7	3·0	20·2	37·1	1·8
5 " . . .	20·6	40·5	6·1	23·7	35·5	6·7
10 " . . .	21·4	33·8	11·4	24·7	37·9	9·8

The tension of the Blood Gases

In order that we may understand the way in which the changes in the blood gases are brought about, it is necessary to describe the known facts respecting their tension.

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[1] P. Bert, *La pression barométrique, &c.* Paris, G. Masson, 1878. *Compt. rend.* vols. lxxiv. and lxxv.

The volume of gas absorbed by a liquid is independent of the pressure; but according to what is known as Boyle's law, the density of a gas, i.e. the number of molecules in a given space, is in proportion to the pressure. Hence, although the volume remains constant, the weight (volume multiplied by the density) of the absorbed gas rises and falls in proportion to its pressure; this is known as the law of Dalton and Henry.

When two or more gases form an atmosphere above a fluid, the absorption takes place in proportion to the pressure which each of the constituents of the mixture would exercise, if it were alone in the space occupied by the mixture; this pressure was termed by Bunsen the *partial pressure* of the gas. Suppose atmospheric air to be under a pressure of 760 m.m. of mercury: the air contains 21 vols. per cent. of oxygen, and 79 vols. per cent. of nitrogen. The partial pressure of the oxygen $=\frac{760\times 21}{100}=159{\cdot}6$ m.m. of mercury; and of the nitrogen $=\frac{760\times 79}{100}=600{\cdot}4$ m.m. of mercury. The carbonic acid of the atmosphere is present in such traces, that its partial pressure is practically zero. Hence when a liquid like soda water, which is charged with carbonic acid, is exposed to the atmosphere, bubbles of the gas escape from the liquid, until the difference of tension or pressure between the carbonic acid in the water and in the air above it, is balanced; the gas which comes off from the liquid exercises, as it does so, a certain amount of pressure; and by the phrase 'tension of a gas in a fluid' is meant the partial pressure in millimetres of mercury, which the gas in question has to exercise in the atmosphere, when no diffusion between the gas in the fluid and the gas in the atmosphere takes place. If the partial pressure of the gas in the atmosphere increases, a greater weight of the gas is absorbed by the fluid; if it diminishes, some of the absorbed gas is given off from the fluid.

We have, however, seen that the formation and dissociation of such compounds as oxyhæmoglobin in the blood is an additional factor to be taken into account, and in point of fact it is found that more oxygen is actually absorbed in the lungs from the alveolar air than can be explained by Dalton's law of pressures. Similarly with regard to the carbonic acid, the compounds which it forms are those which undergo dissociation, and so we have not merely a physical process of gas diffusion from blood to air to deal with.

It will be here a convenient place to explain what is known as *dissociation*. Certain compounds of gases are formed only when the partial pressure of the gas is high. When the partial pressure of the gas is diminished, the constituents of the compound are separated. To express it in more familiar language: when the gas is pressed with force against certain substances, it refuses altogether to combine with some of them; with others it forms so firm a combination that it is exceedingly difficult to separate the gas again from them; and with a third class of substances, those that we are now considering, it forms combinations under protest, as it were, separating from them directly the pressure upon it is reduced sufficiently low. The gas is always tending to separate from the substance with which it is thus loosely combined, and the force which it exercises in this effort is called the *tension of dissociation*; when this is greater than the external pressure of the gas, dissociation takes place.

Applying this now to the blood, we see that the hæmoglobin of the blood in the pulmonary capillaries finds oxygen in the alveoli, which has a comparatively high partial pressure; it therefore unites with the oxygen. In the capillaries of the systemic circulation, the oxyhæmoglobin comes into relation with tissues poor in oxygen, that is, where the partial pressure of the gas is low; the oxyhæmoglobin is dissociated, the oxygen is supplied to the tissues, and the venous hæmoglobin returns to the lungs for a fresh supply of oxygen.

In the case of carbonic acid, compounds are formed in the blood of the tissues where the tension of that gas is high; and in the lungs these undergo dissociation, the gas passing into the alveolar air where the partial pressure or tension of carbonic acid is comparatively low.

The different conditions under which dissociation of oxyhæmoglobin takes place have been recently investigated by Hüfner[1] and by Brasse.[2] Bert showed that dissociation occurs more easily at 40° C. than at temperatures below that point; Frankel and Geppert obtained similar results, and Hüfner has shown that, besides pressure and temperature, another very important factor is the concentration of the solution of oxyhæmoglobin used; so that in the body the amount of oxyhæmoglobin in the red corpuscles must be taken into account.

At high temperatures, as in the blood during fever, the conditions under which oxyhæmoglobin is dissociated are therefore different from those which obtain in health.

Hüfner calculates that at a height above the sea-level of over 5,500 metres the atmospheric pressure and the partial pressure of oxygen are so low that oxyhæmoglobin would be dissociated, and thus such an elevation would be exceedingly perilous to animal life. This coincides very well with the results obtained by actual experience. He suggests, however, that breathing would be still possible at such altitudes, by increasing the richness of the blood in hæmoglobin ; this may be done by transfusing more blood into the vessels.

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[1] *Zeit. physiol. Chem.* xii. 568; xiii. 285.

[2] *Compt. rend. Soc. biol.* v. 660.

The researches of L. Brasse relate chiefly to the influence of temperature on the dissociation of oxyhæmoglobin. He finds that the tension of dissociation for oxyhæmoglobin at 0° C. is *nil*. The compound is thus a stable one at that temperature ; in hibernating animals, the temperature of the body is very low, and the blood is red in the veins as well as in the arteries. The tension of dissociation increases with the temperature, and a mammal dies when the temperature of its blood reaches 45° C. At this temperature, although the tension of dissociation is still lower than the partial pressure of the atmospheric oxygen, it is higher than that of the oxygen in the pulmonary alveoli. In birds, on the other hand, where by the arrangement of air sacs the aëration of the blood is very complete, they do not die until their blood reaches the temperature of 50° C.

The tension of the gases is thus the sum of the tension of dissociation of the oxyhæmoglobin, bicarbonates, and similar compounds, with the physical tension of the small amount of the gases dissolved in the blood plasma. The tension (composed of these two factors) of the gases in the blood is not nearly so great as it would be if all the gas in the blood were in a free or uncombined condition. The measurement of the tension of the gases in the blood was carried out by Pflüger,[1] and his pupils Wolffberg,[2] Strassburg,[3] and Nussbaum [4] ; the instrument they used is called an aërotonometer.[5]

The average results obtained may be thus summarised (Strassburg) :—

Tension of oxygen in arterial blood=29·64 mm. of mercury=3·9 per cent. of an atmosphere.

Tension of oxygen in venous blood=22·04 mm. of mercury=2·9 per cent. of an atmosphere.

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[1] *Pflüger's Archiv*, vi. 43.

[2] *Ibid.* iv. 465 ; vi. 23.

[3] *Ibid.* vi. 65.

[4] *Ibid.* vii. 296.

[5] The use of this instrument may be best explained by an example. Suppose that one wished to ascertain the tension of carbonic acid in the blood ; the blood direct from the living vessels is introduced into the upper end of a vertical glass tube (kept at a constant temperature by a jacket of water) containing nitrogen and a small known percentage of carbonic acid. The blood runs down the tube and is at once removed at the lower end, means being provided to prevent air getting to it. If the tension of carbonic acid in the blood is greater than in the mixture in the tube, then the amount of carbonic acid in the tube will be increased after the blood has passed through it ; if the tension in the blood is less, then the amount of carbonic acid in the tube will be found to be diminished. By successive experiments it is found that for a certain percentage the amount of carbonic acid undergoes no change ; this percentage therefore exerts the same tension as the carbonic acid in the blood. Strassburg (*loc. cit.*) gives a figure of the aerotonometer.

Tension of carbonic acid in arterial blood = 21·28 mm. of mercury = 2·8 per cent. of an atmosphere.

Tension of carbonic acid in venous blood = 41·04 mm. of mercury = 5·4 per cent. of an atmosphere.[1]

The differences in the tension of the gases is thus much less than the differences in their volume, in the two varieties of blood.

Let us now compare the tension of the blood gases with the partial pressure of the gases in the pulmonary alveoli. Wolffberg obtained the residual or alveolar air from the lungs of dogs by catheterisation, and the following are his mean results :—

Tension of oxygen in alveolar air = 27·44 mm. of mercury = 3·6 per cent. of an atmosphere.

Tension of carbonic acid in alveolar air = 27·06 mm. of mercury = 3·56 per cent. of an atmosphere.[2]

Now if the line AB in the accompanying diagram represents the alveolar membrane, there is the alveolar air on one side of it, and venous blood on the other ; the tension of the two gases in each is represented in the diagram, and the direction in which diffusion takes place is shown by the arrows ; the oxygen passing from alveolar air into the blood, the carbonic acid in the reverse direction.

$$\text{Alveolar air}\quad \begin{array}{ll} & \text{Tension} \\ O\,; & 27{\cdot}44 \\ CO_2\,; & 27{\cdot}06 \longleftarrow \end{array} \begin{array}{c} A \\ | \\ | \\ B \end{array} \begin{array}{ll} \text{Tension} & \\ \longrightarrow 22{\cdot}04\,; & O \\ 41{\cdot}04\,; & CO_2 \end{array}\quad \text{Venous blood}$$

It has long been felt that the comparatively small differences of partial pressure (particularly of oxygen) do not completely explain the very great differences in the volume of the gases in arterial and venous blood, and any account of the gases of the blood would be incomplete without a reference to the ingenious theory recently advanced by Ernst Fleischl v. Marxow.[3] The author, after stating the usual theory of respiration and its difficulties, asks how it is that, if the tissues have a greater affinity for oxygen than hæmoglobin, the blood of animals killed by asphyxia still contains a considerable amount of oxyhæmoglobin ; and v. Marxow believes that in the sharp, sudden stroke of the heart's beat he has discovered a physical agency which assists in the work of dissociation ; according to him the blood is kept in motion by a series of quick sudden strokes, because for the taking up of the oxygen by the tissues, and the elimination of carbonic acid by the lungs, it is not sufficient that the blood runs steadily through the systemic and pulmonary capillaries respectively ; and therefore a short, hard stroke is given to it immediately before it enters, and immediately after it has left the lungs; the systole of the left ventricle assisting in the liberation of the oxygen; of the right ventricle in the liberation of the carbonic acid. That a blow has very considerable power in assisting the liberation of gases can be readily demonstrated with an ordinary hypodermic syringe; if the piston be pulled up, and water allowed to rush into the vacuum so formed, bubbles of gas will come off from the water; but if the handle of the piston first receives a sharp blow from a mallet, the gas bubbles will come off so rapidly that the water froths.

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[1] This rose on the coagulation of the blood to 61·79 mm. Hg, = 8·13 per cent. of an atmosphere.

[2] Nussbaum obtained rather a higher number, 29·18 mm. Hg.

[3] *Die Bedeutung des Herzschlages für d. Athmung ; eine neue Theorie der Respiration*, Vienna. I am indebted to Prof. McKendrick's address to the Brit. Med. Assoc. 1888 (*Brit. Med. Journ.* August, 1888) for the above abstract of v. Marxow's theory.

Although physiologists cannot but treat with the greatest respect the conclusions arrived at by so eminent a physicist as Fleischl von Marxow, it must be admitted that there are many difficulties in the way of fully accepting his theory in its entirety. These difficulties are chiefly the two following:—

(1) In small mammals the stroke of the heart cannot be nearly so powerful as in large mammals; but still the same respiratory exchanges go on.

(2) In cold-blooded animals there is only one ventricle, and the blood receives only a single blow; but, nevertheless, on its way from the heart back to the heart again it undergoes two gaseous exchanges, first in the lungs or gills, secondly in the tissues.

In spite of these obvious objections, which show that v. Marxow is inclined to exaggerate the importance of the heart beat, it is, however, quite possible that in the warm-blooded animals, where the gaseous exchanges are more extensive than in the cold-blooded animals, the force of the blow given to the blood by the heart may exercise some auxiliary impulse in the liberation of the blood gases.

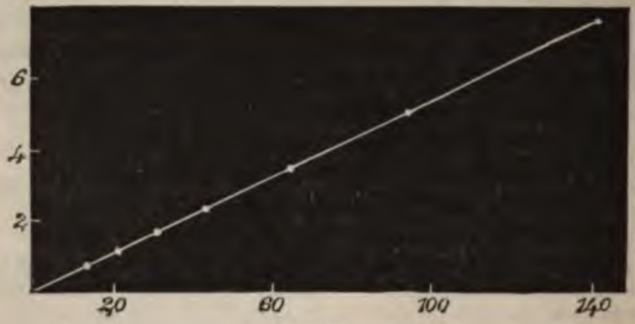

FIG. 66.

Another attempt to elucidate the perplexing questions involved in the respiratory exchange of gases has been recently made by Christian Bohr. We have already seen that some of the carbonic acid is contained in the red corpuscles, and Bohr considers that it is in actual combination with the hæmoglobin; he considers that this union is like oxyhæmoglobin—a dissociable one—and that dissociation takes place in the pulmonary alveoli. If this is really the case, hæmoglobin appears to be not only an oxygen carrier but also a carbonic acid carrier. We have, of course, in addition to this, the carbonic acid dissolved in the plasma, in the form of carbonates and bicarbonates. Bohr's theory of the combination which occurs in the red corpuscles appears to me so important and full of interest that I propose here to give a brief résumé of his paper[1]:—

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[1] Ludwig's *Festschrift*, 1887, p. 164.

Setschenow,[1] and later Zuntz,[2] stated that a solution of hæmoglobin at the atmospheric pressure absorbs more carbonic acid than the same volume of water. Bohr's research was devoted to studying this subject more fully, and to ascertaining the relation between the tension of the carbonic acid and the amount absorbed per gramme of hæmoglobin. A special absorptiometric method employed was described by him in an earlier paper.[3] Pure solutions of crystalline hæmoglobin from the dog, and pure carbonic acid, were employed; these were brought in contact with one another, the gas being at a known pressure, and the temperature kept constant throughout. The amount of gas absorbed was afterwards pumped off and estimated. Some preliminary experiments were made with water; the result of one of these, in which 41 grammes of water were used, may be represented graphically as in fig. 66. The line which indicates the increase of absorption is constructed from ordinates representing the amount of absorbed gas in grammes, the abscissæ the pressure of the gas in mm. of mercury. As is seen, the result is a straight line, the weight of gas absorbed being proportional to its tension (Dalton-Henry law).

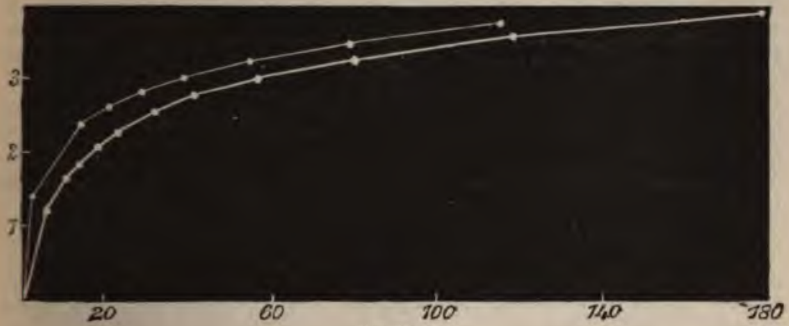

Fig. 67.

Experiments were then made with hæmoglobin solutions; the following table represents a portion of one of these:—

Pressure of CO_2 in mm. of Mercury	Total quantity of CO_2 absorbed	Physical Absorption; i.e. the CO_2 which would be absorbed by an equal quantity of water	Amount of CO_2 absorbed per 1 gramme of Hæmoglobin	Temperature
6·04	2·0975	0·275	1·269	18·2° C
11·57	2·8847	0·527	2·358	18·4°
14·62	3·2295	0·666	2·564	18·4°
18·54	3·6656	0·844	2·822	18·4°
24·07	4·1966	1·095	3·102	18·4°
31·98	4·8548	1·455	3·400	18·4°

The quantity of carbonic acid absorbed by hæmoglobin is thus immensely greater than that explicable on simply physical grounds. The curves in fig. 67

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[1] *Mém. de l'acad. de St. Pétersbourg*, vol. xxvi. 1879.

[2] Hermann's *Handbuch*, vol. iv. 2. p. 76.

[3] Bohr, *Exper. Untersuch. ü. d. Sauerstoffsaufnahme des Blutfarbstoffs*, Kopenhagen, 1885.

represent graphically two experiments; the abscissæ represent as before the pressure, the ordinates the quantity of gas absorbed per gramme of hæmoglobin. The curve is very different from the straight line of fig. 66, and the ascent of the curve is especially steep at the lower pressures. The upper curve is the representation of an experiment performed with a less concentrated solution of hæmoglobin than in the experiment represented by the lower curve. It thus appears that the amount of gas absorbed is less in the more concentrated solution. Contrasting the curves with those obtained in experiments with other gases (oxygen, carbonic oxide, nitric oxide) which are known to form compounds with hæmoglobin, they are found to be different. Hence, if we have to do with a chemical union of carbonic acid and hæmoglobin, the gas is combined differently from that in oxyhæmoglobin, CO-hæmoglobin, and NO-hæmoglobin respectively. The spectrum of CO_2 hæmoglobin has still to be investigated.[1] S. Jolin[2] repeated these experiments with guinea-pig's hæmoglobin, and obtained similar results. With birds' hæmoglobin the curves were rather different, both in respect to oxygen and carbonic acid.

TISSUE RESPIRATION

Our present knowledge concerning tissue respiration has been necessarily dealt with in our consideration of the gases of the blood. It will be, however, here interesting to add a few historical points in connection with this subject.[3]

According to Lavoisier, respiration was considered to be a slow combustion of carbon and hydrogen; the air supplied the oxygen, the blood the combustible materials. The great French chemist's opinions were however much misunderstood, and a notion prevailed that, according to him, combustion occurred only in the lungs; that these organs in fact acted as a stove for the remainder of the body. Lagrange a few years later (1791) clearly pointed out how impossible this was, for if all the heat of the body were produced in the lungs alone, their temperature would be raised so high as to destroy them; he therefore supposed with Lavoisier that the oxygen dissolved in the blood combined there with carbon and hydrogen to form carbonic acid and water respectively.

The next step was the discovery by Spallanzani that animals confined in an atmosphere of nitrogen or of hydrogen exhaled carbonic acid to almost as great an extent as if they had breathed air; he supposed that the carbonic acid was formed by digestion in the stomach, then passed through the tissues, and was finally exhaled. He thus missed a great step in the discovery, namely, that the carbonic acid is produced in the tissues themselves. It was, however, pointed out by W. Edwards[1] that the quantity of the gas was too great to be accounted for in the way Spallanzani supposed; and in 1830, Collard de Martigny stated that carbonic acid was secreted in the capillaries, and excreted by the lungs. The difficulty felt by the older physiologists in accepting the secretion theory was the absence of proof of the existence of free oxygen and carbonic acid in the blood. This proof has been since supplied, and the knowledge supplemented by that concerning the office that hæmoglobin fulfils as oxygen carrier.

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[1] S. Torup (*Maly's Jahresb.* xvii. 115) states that the band of CO_2 hæmoglobin is almost indistinguishable from that of hæmoglobin, but careful measurements show that its darkest part is rather nearer the violet end of the spectrum in the former than in the latter. This observation, however, still awaits confirmation.

[2] *Du Bois Reymond's Archiv*, 1889, p. 265.

[3] For these I am indebted to Dr. McKendrick's address already quoted.

The high tension of carbonic acid in the tissues has already been mentioned as the cause of the formation of bicarbonates, and similar compounds; the comparatively low tension of that gas in the air-cells of the lungs leads to the dissociation of these compounds, and the carbonic acid so liberated passes into alveolar air, and ultimately into the atmosphere. Strassburg[2] found that the tension of carbonic acid in the intestinal walls was 7·7 per cent. of an atmosphere; that is, higher than in venous blood, where it is 5·4 per cent. of an atmosphere; in lymph, bile, urine, &c., it is also higher than in the blood, though oxygen is practically absent.

It is therefore the tissues, not the blood, that become primarily loaded with carbonic acid, the latter simply receiving the gas from the former; the oxygen taken from the blood by the tissues does not immediately proceed to form carbonic acid with the carbon, and water with the hydrogen; but it is taken up by the tissue and held in reserve in some combinations from which it is no longer removable by diminution of tension.

The ultimate processes of respiration have in course of time been transferred from the lungs to the blood, and thence to the tissues. In so far as the lungs and the blood are tissues, they of course contribute their share, but not in greater proportion to that of the other living constituent elements of the body. Blood when shed has little or no oxidising power. Dextrose and uric acid when mixed with it remain unaltered (Hoppe-Seyler).[3] A. Schmidt and Ludwig[4] searched for reducing substances in the blood, but found them only in the blood of asphyxia. Ehrlich,[5] on the other hand, has found reducing substances in most tissues, alizarin blue and other blue pigments losing their colour in the living tissues; these turn blue again in contact with the air.

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[1] *Influence of Physical Agents in Life*, 1823. More recently Pflüger (*Pflüger's Archiv*, x. 251) has shown that a frog at a low temperature will live for hours producing carbonic acid in an atmosphere free from oxygen.

[2] *Pflüger's Archiv*, vi. 65.

[3] *Med. Chem. Unters.* p. 136.

[4] *Ber. der sächs. Ges. d. Wiss. Leipzig. Math. phys. Classe*, xix. 99.

[5] *Der Sauerstoffbedürfniss des Organismus*, Berlin, 1885.

THE GASES OF LYMPH, CHYLE, AND SIMILAR FLUIDS

The following analyses of the gas of the lymph of the dog were made b Hammarsten[1]:—

	Oxygen in vols. per cent.	Carbonic Acid	Nitrogen
Lymph from the left fore leg, 3 observations	0·0, 0·1, 0·0	41·8, 47·1, 44·0	1·1, 1·5, 1·2
Lymph from the thoracic duct .	0·1	37·5	1·63

Oxygen is thus absent, or only present in traces; nitrogen is present in about the same amount as in blood; the quantity of carbonic acid is greater than in arterial and less than in venous blood.

Tschiriew[2] pursuing the investigation made comparative analyses of the lymph, the blood, and the serum of the same animal, and obtained similar results; Buchner[3] found that in asphyxia, as the quantity of carbonic acid in the blood increased, that in the lymph diminished.

In these researches most interest is attached to the amount of carbonic acid present; and from them it might be argued that the formation of carbonic acid takes place within the blood vessels rather than in the tissues, which is the direct contrary of what really is the case. No doubt the tension of carbonic acid gas is greater at its seats of formation, i.e. near the anatomical elements of the tissues, than it is in the lymph; and if we analyse the normal secretions, bile, saliva, &c., which result more directly from the action of the cells, we shall find that the tension of carbonic acid in them is higher than in lymph, higher than in venous blood, and probably represents very accurately the gaseous tension at the actual seat of respiratory combustion. Gaule[4] also has shown that, though the quantity of carbonic acid in the lymph is less than that in the serum, its tension is higher. The same difference doubtless holds between the tension of that gas in the lymph and plasma, and so its passage from the former into the latter is easily accounted for.

The following are some analyses of the gaseous contents of certain secretions, which bear out the statement just made:—

Secretion	Oxygen	Carbonic Acid			Nitrogen	Observer
		Removable *in vacuo*	Removable by Acid	Total		
Bile	0·2	14·4	41·7	56·1	0·4	Pflüger[5]
„	—	19·5	37·0	56·5	—	Bogoljubow[6]
„	—	17·1	62·5	79·6	—	„
Submaxillary Saliva (dog)	0·4	19·3	29·9	49·2	0·7	Pflüger[7]
	0·6	22·5	42·2	64·7	0·8	„
Parotid Saliva (human)	1·0	3 to 5	40 to 60	43·5–63·5	2·5	R. Kulz[8]

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[1] *Ber. d. Gesellsch. d. Wiss.* Leipzig, xxiii. 617.

[2] *Ibid.* xxvi. 38.

[3] Ludwig's *Arbeiten*, 1876.

[4] *Ibid.* 1878; *Du Bois Reymond's Archiv*, 1878, p. 469.

[5] *Pflüger's Archiv*, ii. 173.

[6] *Cent. med. Wiss.* 1869, No. 42.

[7] Heidenhain's *Studien des physiol. Inst. Breslau*, Leipzig, Heft iv. p. 25.

[8] *Zeit. Biol.* xxiii. 321.

The following table represents the composition of the gaseous contents of various pathological transudations :—

Fluid	Oxygen	Carbonic Acid			Nitrogen	Observer
		Removable *in vacuo*	Removable by Acid	Total		
Peritoneal	0·139	9·404	4·866	14·27	2·107	Planer[1]
Hydrocele	0·16	32·49	32·45	64·94	2·05	Strassburg[2]
Subcutaneous œdema	traces	22·25	9·11	31·36	traces	Ewald[3]
„ „ (case of nephritis)	„	21·88	31·18	53·06	„	„
Pleuritic	0·68	39·34	15·59	54·93	1·33	„
„ (case of phthisis)	0·54	18·54	25·99	44·53	1·87	„
„	—	18·64	41·16	59·80	—	„
„	0·17	25·47	46·82	72·29	1·04	„
Hydrothorax . . .	0·29	25·34	48·67	74·01	0·87	„
„ . . .	1·01	25·71	55·50	81·21	2·47	„

Ewald also by the use of Pflüger's aërotonometer estimated the tension of carbonic acid in four of these fluids; his results were respectively 7·51, 10·92, 10·73, and 11·5 per cent. of an atmosphere. Thus not only the quantity but also the tension of this gas is greater in these transudations than in venous blood.

The gases of pus have also been analysed by Ewald ; the following table gives a summary of his results :—

Nature of Pus	Carbonic Acid			Oxygen	Nitrogen
	Removable *in vacuo*	Removable by Acid	Total		
Empyema, 28 days standing	70·17	1·68	71·85	1·14	
„ 10 „ „	15·73	2·77	18·50	traces	
„ „ „ „	14·76	?	14·76	„	
„ „ „ „	21·46	0·0	21·46	2·9	0·77
Abscess, 21 „ „	8·05	0·0	8·05	1·35	0·43
„ „ „ „	7·92	0·0	7·92	—	

The amount of carbonic acid present increases with the age of the exudation ; the more nearly the purulent exudation approaches pure pus in its characters the smaller is the total carbonic acid, and especially the more firmly combined carbonic acid ; indeed, pure pus contains only loosely-combined carbonic acid. It is very probable that the pus corpuscles, in common with the blood corpuscles, possess the power of decomposing sodium carbonate (Na_2CO_3) and driving off carbonic acid from it.

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[1] *Zeit. d. Gesell. d. Aerzte in Wien*, 1859.

[2] *Pflüger's Archiv*, vi. 94.

[3] *Archiv für Anat. u. Physiol.* 1873, p. 663 ; 1876, Heft iii.

CUTANEOUS RESPIRATION

The greater part of the respiratory exchange of gases occurs through the [illegible] membrane of the pulmonary alveoli. A certain amount of gaseous exchange occurs also through the thicker mucous membranes of the respiratory tract, and also through the skin. The amount of cutaneous respiration varies in different animals, but is greatest in those in which the epidermis is thinnest, and thus presents the least resistance to the diffusion of gases. In frogs, for instance, where not only is the skin thin, but it has a rich blood supply, Regnault and Reiset found that nearly as much oxygen was used and carbonic acid given off after extirpation of the lungs as before that operation. The following are the results of Fabini's[1] analyses:—

Healthy frogs in the light . . .	632	milligr. of CO_2 per 100 grms. of body weight in the 24 hours
Frogs without lungs in the light .	569	„ „ „
Frogs without lungs in the dark .	424	„ „ „

The amount of cutaneous respiration in man has been discovered by enclosing a portion of the body, such as a limb, in an air-tight bag, and after a time analysing the gases (oxygen and carbonic acid) contained therein. From this the amount occurring over the whole cutaneous surface can be calculated. In some experiments the whole body was enclosed in an air-tight chamber. The following numbers give the quantity in grammes of carbonic acid which passes out through the whole skin of a man in the 24 hours, according to different observers: Abernethy,[2] 14; Scharling,[3] 32; Gerlach,[4] 8·5: Reinhard,[5] 2·2; Aubert and Lange,[6] 3·8; Rohrig,[7] 14; Fabini and Ronchi,[8] 6·8. The last-named observers also found that the quantity of carbonic acid increased with a rise of atmospheric temperature. The amount of carbonic acid excreted by the skin of warm-blooded animals is so small that death which follows varnishing the skin cannot be accounted for by the stoppage of this function.

FŒTAL RESPIRATION

The fœtus receives its supply of oxygen from the maternal blood; the gaseous exchanges occur through the thin walls of the vessels of the placenta; other nutritive materials pass in a similar way from mother to the embryo. The difference between the arterial and venous blood of the fœtus is not nearly so marked as in extra-uterine life.[9] The need of the fœtus for oxygen is much less than it is after birth, and the amount it receives from the maternal blood is not only ample for its wants, but is sufficient to maintain a condition of apnœa.

The respiratory changes occurring in hens' eggs during incubation have been studied by Baumgärtner.[1] The apparatus used was constructed on the principle of that of Regnault and Reiset. While the egg is in the condition of rest, no metabolic changes occur; but when incubated, oxygen is absorbed, and carbonic acid given off. In twenty days' incubation 1755·3 c.c. of oxygen were absorbed, and 1626·2 c.c. of carbonic acid given off; the respiratory quotient was therefore 0·927.

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[1] J. Moleschott, *Unters. z. Nahrlehre d. Menschen*, xii. 100 (1878).

[2] *Surgical and physiol. Essays*, London, 1793–7.

[3] *J. prakt. Chem.* xxxvi. 455.

[4] *Müller's Arch.* 1851, p. 453.

[5] *Zeit. Biol.* v. 33.

[6] *Pflüger's Arch.* vi. 539.

[7] *Deutsche Klinik*, 1872, p. 209.

[8] J. Moleschott, *Unters. z. Nahrlehre d. Menschen*, xii. 100 (1878).

[9] The presence and amount of oxyhæmoglobin and hæmoglobin in fœtal blood has been specially studied by Zweifel (*Arch. f. Gynäkol.* xi. Heft ii. p. 1) and Zuntz (*Pflüger's Archiv*, xiv. 605).

RESPIRATION IN FISHES

We have hitherto considered respiration in air-breathing animals only; it is now necessary to briefly describe the process as it occurs in fishes and other aquatic animals.

Munk[2] made a number of comparative experiments in which he contrasted what may be called the intensity of respiration; that is, the amount of oxygen in grammes used per kilogramme of body weight per hour. His results are given in the following table:—

Animal	Intensity of Respiration	Respiratory Quotient $\frac{CO_2}{O_2}$
Cat	1·007	0·77
Dog	1·183	0·75
Rabbit	0·918	0·92
Hen	1·300	0·93
Small singing birds	11·360	0·78
Frog	0·084	0·63
Cockchafer[3]	1·019	0·81
Man	0·417	0·78
Horse	0·563	0·97
Ox	0·552	0·98
Sheep	0·490	0·98

The intensity of respiration is exceptionally high in small birds; in the frog, which may be taken as an example of a cold-blooded animal, it is very low; the same is true for fishes. This is in consonance with the fact that sea water contains only small quantities of oxygen. The sea water brought home by the *Challenger* expedition was analysed by Prof. Dittmar[4]; he says: The ocean can nowhere contain more than 15·6 c.c. of nitrogen and 8·18 c.c. of oxygen per litre. The nitrogen never falls below 8·55 c.c.; but the theoretical minimum of oxygen (4·3 c.c.) is liable to diminution by the processes of life and putrefaction; and as a matter of fact water from a depth of 1500 fathoms gave 2·04 c.c., and from a depth of 2875 fathoms 0·6 c.c. per litre, and yet many forms of life exist at this great depth. Fishes have been dredged from a depth of 2750 fathoms, where the amount of oxygen was probably not so much as 0·06 c.c. per 100 c.c., or 300 times less than that in the arterial blood of a mammal. The amount of oxygen in the blood of a fish is less than in that of a mammal, but it still contains much more oxygen than exists in the same volume of sea water. The water is, however, constantly renewed, and the mechanism by which thin sheets of water are propelled over the gills was first fully described by Flourens.[1] Aquatic breathers are not, however, troubled with free carbonic acid. This was shown by the *Challenger* chemists to be the case, because any carbonic acid formed is at once absorbed by the excess of alkaline bases present in the water.[2] There is thus no tension of carbonic acid in the water to prevent or hinder its escape.

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[1] *Der Athmungsprocess im Ei*, Freiburg, 1861; *see* also Pott, *Maly's Jahresb.* 1877, p. 328. *Pflüger's Archiv*, xxxi. 268.

[2] I. Munk, *Physiol. des Menschen und der Säugethiere*, 1888, p. 82.

[3] Insects thus take as much oxygen in proportion to their weight as mammals; this was previously known from the researches of Regnault and Reiset.

[4] *Proc. Phil. Soc. Glasgow*, xvi. 61.

Oxygen also is probably stored in the swimming bladder; and this presumably oxygenates the blood when the fish dives into the almost airless depths of the ocean. Thus Biot[3] found 70 vols. per cent. of oxygen in the swimming bladders of such fishes, a gas in which a glowing splinter of wood is rekindled. Other observers have, however, shown that in fishes living near the surface of water the quantity of oxygen is much less in their swimming bladder. The following analyses have been made :—

	Oxygen	CO_2	Nitrogen	
Carp . . .	10·7 – 7·1 per cent.	5·2	87·7	(Humboldt and Provençal)[4]
Cyprinus .	13·2 – 1·1 „	1·4–3·9	80·8–97·5	(Fr. Schultze)[5]

Margareta Traube Mengarini[6] has shown that, if hydrogen is dissolved in the water, that gas soon appears in the swimming bladder even of those fishes whose swimming bladder is a closed one.

Humboldt and Provençal were the first who made quantitative estimations of the respiratory exchanges in fishes; more complete observations were made by Baumert,[7] and a very exhaustive study of the process in both fresh-water and sea-water fishes and other aquatic animals has been made by Jolyet and Regnard.[8] The following examples may be taken from their tables :—

Fresh-Water Animals	Temperature of Water	Amount of Oxygen taken in per kilog. of body weight in one hour		Respiratory Quotient $\frac{CO_2}{O_2}$
		In c.c.	In grammes[10]	
Cyprinus tinca	14° C.	57·7	0·082	0·66
Muræna anguilla . . .	14°–15°	40·5–48·0	0·058–0·068	0·6–0·79
Cobitis fossilis	17°–22°	86·3	0·123	0·78
Axolotl	11·5°	45·2	0·064	0·56
Astacus fluviatilis . . .	12·5°	38·0	0·054	0·86
Hirudo officinalis . . .	13·5°	23·0	0·032	0·69
Sea-Water Animals				
Mullus	14°–15°	134–171	0·191–0·244	0·81–0·86
Muræna conger	13°–16°	59·8–75·5	0·085–0·109	0·67–0·72
Pleuronectes solea . . .	14°	73·5	0·105	0·81
Cancer	16°	107·0	0·152	0·85
Homarus	15°	68·0	0·097	0·8
Octopus vulgaris . . .	15°–16°	44·	0·0628	0·65–0·86
Mytilus edulis[9]	14°	12·2	0·017	0·76
Asteracanthion rubens .	19°	32·0	0·045	0·79

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[1] *Mémoires d'anatomie et de physiologie comparées*, Paris, 1844, p. 75.

[2] This is the principle of Fleuss' diving apparatus.

[3] *Ann. d. Chem. u. Physiol.* 1808, iv. 582.

[4] *Mémoires de phys. et de chim. de la soc. d'Arcueil*, ii. 359.

[5] *Pflüger's Archiv*, v. 48.

[6] *Du Bois Reymond's Arch.* 1889, p. 54.

[7] *Chem. Unters. über die Resp. des Schlammpeizgers*, Heidelberg, 1852.

[8] *Maly's Jahresb.* 1877, p. 332.

[9] Weight of shell included in body weight.

[10] This table gives what was called the intensity of respiration in the table p. 395.

It is very necessary to note the temperature of the air or water when making observations on cold-blooded animals, since the temperature of the animal's body, and therefore its chemical activity, rises and falls with that of the surrounding medium.

The following example from Jolyet and Regnard may be given in illustration:—

Animal	Temperature of Water	Oxygen in c.c. absorbed per hour per kilo of body weight	Respiratory Quotient
Cyprinus auratus . . .	2°	14·8	0·89
The same animal . . .	10°	37·8	0·96
" " . . .	30°	147·8	0·75

The death of fishes when placed in the air appears to be due, not to the drying of the gills, but to the large excess and high partial pressure of oxygen.

CHAPTER XX

MUSCLE

INTRODUCTORY

Muscle is a tissue which may be very conveniently considered next to blood, as in many points a resemblance between it and blood is to be noted.

Microscopically examined, muscular tissue is found to be made up of bundles of fibres, and these fibres which are really elongated cells differ in structure in the voluntary and involuntary muscles.

The voluntary muscles, i.e. the skeletal muscles, are composed of transversely striated fibres. The involuntary muscles, with the exception of cardiac muscle, are made up of spindle-shaped fibres, which exhibit no transverse markings, and are often called plain or unstriated muscular fibres. The cardiac muscular fibres are striated, but exhibit certain marked histological differences from the voluntary fibres.

Not only do muscular fibres differ histologically, there are also physiological differences, which may be roughly summed up as follows: (1) the latent period of involuntary muscle is much longer, and the contraction slower, than in the case of the voluntary muscles; (2) the contraction of involuntary muscles is either rhythmical, as in the heart, or has a tendency to become so, as in the uterus, alimentary canal. The contraction, moreover, passes as a wave from one muscular fibre to another, and thus the movement known as peristalsis is produced.

What, however, concerns us more especially is not the histology of muscular fibres, nor the physical conditions of their contraction, but the differences in their chemical composition and in their chemical changes. Here, however, we are not able to speak so positively; nearly all our knowledge of the chemistry of muscle is derived from the study of voluntary muscle; a much more limited number of observations have been made on the involuntary muscles. Such observations as have been made on the involuntary muscles show that speaking generally they have the same composition as the voluntary muscles, and the changes they undergo during contraction are very similar to those occurring on the contraction of voluntary muscular fibres; the chemical changes, however, like the physical changes during contraction, are not so vigorous.

In our consideration of muscular tissue we shall have to study, first, what can be learnt of its chemical structure by means of such instruments as the microscope and polariscope; secondly, the general composition and enumeration of the constituents of the tissue; next it will be necessary to take up certain of these constituents and discuss them at greater length, especially the proteids, the pigments, the extractives, the salts, and the gases of muscle. Lastly, it will be necessary to consider the changes which muscle undergoes when it contracts, and when it dies (*rigor mortis*), and the influence of muscular activity upon the rest of the body, particularly on the blood which is circulating through the muscles, and on the composition of the expired air and the urine.

MICROSCOPIC STUDY OF MUSCULAR FIBRES

Voluntary muscular fibres.—These are bound into bundles (fasciculi) by means of areolar tissue. The fibres themselves are cylindrical, or approximately so; they vary in diameter from $\frac{1}{750}$ to $\frac{1}{400}$ of an inch in the muscles of the trunk and limbs, and from $\frac{1}{750}$ to $\frac{1}{2400}$ of an inch in the muscles of the head and face (Kölliker); as a rule they are not branched; in length they do not generally exceed an inch and a half, but in some long muscles, like the sartorius, they are considerably longer. Each fibre consists of an external sheath that is called the *sarcolemma* or *myolemma*; this encloses the contractile substance. The sarcolemma is transparent, elastic, and apparently homogeneous; it resists the action of acetic acid and of boiling water; it is, however, by prolonged treatment with these reagents ultimately dissolved. Hence, although it resembles elastin in insolubility, it is not so insoluble as the elastin of elastic tissue. Beneath the sarcolemma are found a number of oval, flattened nuclei, surrounded by a small amount of granular protoplasm; these nuclei, derived from the multiplication of the nucleus of the original cell from which the fibre was developed, are rendered evident by treatment with acetic acid; they consist, as nuclei do generally, of a network of fibres, but the transverse meshes are especially well marked. Very little nuclein is, however, obtainable from muscular tissue (*see* also p. 204). It is the contractile substance proper within the sarcolemma that has the striated appearance typical of this variety of muscular tissue. At first sight there are alternate layers or discs of light and dark substance; on closer examination an intermediate dotted stripe is seen in the centre of the light stripe, which has been called a membrane (Krause's membrane), or Dobie's line; by varying the focus the line appears double. Insects' muscle is very highly developed, and has been largely used for microscopic study; in this muscle the dark stripe appears to consist of a number of rods of which the long axis is the same as that of the muscular fibres, and the dots of the intermediate stripe appear to be knobs at the extremities of the rods (Schäfer). In contraction, the light and dark stripes apparently change places. There is no doubt that the optical conditions vary much with the focus of the microscope, and thus the subjective effects produced by the examination of the tissue are largely accounted for; a number of theories as to the plan of construction of a muscular fibre have therefore arisen. For a full description and discussion of these various theories the reader is referred to works on histology. What, however, seems to be certain is this:—

1. The contractile portion of the muscular fibre is a semi-fluid material like protoplasm. Kühne and Eberth observed a minute nematoid worm (Myoryctes Weissmanni) moving in the interior of living muscular fibres in the frog, and noticed that the transverse striæ and other markings displaced by its movement closed in again behind it, reassuming their former order and position.

2. The various optical appearances are produced by the existence of two distinct substances in the contractile portion of the muscle. Neither of these has more than a semi-solid consistency; still, one of them appears to be more solid than the other. The more solid substance is that which forms the structures variously described as rods, knobs, membranes, discs, fibrils, &c., and the less solid material is that in which the more solid structures are suspended.

3. Examined in the dark field of the polarising microscope (see p. 38) the more solid substance remains dark, and is thus isotropous or singly refracting; the less solid substance is bright, that is to say, it is anisotropous or doubly refracting.

4. By certain artificial means, e.g. by weak hydrochloric acid, a muscular fibre can be split into discs; this separation occurs at the intermediate dotted line.

5. By certain other reagents, e.g. alcohol, a muscular fibre can be split longitudinally into fibrils, indications of which can be seen as faint longitudinal markings in the healthy fibre.

6. Supposing these two operations to take place simultaneously, a muscle can be finally subdivided into a number of approximately cubical blocks (Bowman's sarcous elements).

The most recent investigations on the subject of the structure of the contractile substance of muscular tissue are those in which gold chloride has been chiefly used as a staining reagent (Retzius,[1] Melland,[2] Marshall,[3] van Gehuchten[1]). These teach that the isotropous material is a network of fine fibrillæ pervading the whole of the contractile substance; the interstices between the fibrillæ are filled up by the less solid, doubly-refracting substance.

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[1] *Sitzungsb. d. Wiener Akad.* 1881.

[2] Melland, *Quart. J. of Microscop. Science*, vol. xxiv. p. 371 (1885).

[3] Marshall, *Ibid.* vol. xxvi. (1887), p. 75; *ibid.* April 1890.

A typical animal cell is a mass of protoplasm containing a nucleus. It may or may not have a cell wall; it generally has not. The nucleus consists of a network of nucleoplasmic fibres, and a nuclear matrix, a homogeneous substance that pervades the whole nucleus; the protoplasm of the cell also contains a network of fine fibrillæ, and the unfibrillated stroma in which this fibrillar network is situated is called the enchylema (Carnoy).

A muscular fibre is an animal cell; each one is developed from a typical animal cell; the fully-formed muscular fibre is, however, an animal cell which has become specialised in certain points both of structure and action; it possesses, like protoplasm, contractility, but its contractility does not come into play so as to produce movements in all directions, as in the amœba or white blood corpuscle, but is limited so as to produce shortening in one direction only; then in structure it is a cell which has become elongated, and of which the nuclei have increased in number and become peripheral in position; it is a cell with a well-marked cell wall, the sarcolemma; and, lastly, it is a cell in which the fibrillar network is no longer irregular, but is arranged with

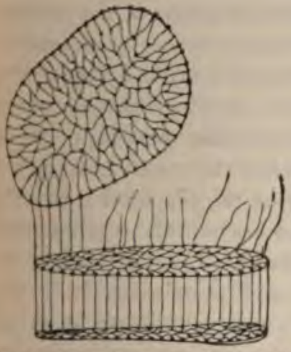

FIG. 68.—Part of a muscular fibre of Water-beetle. The fibre has been prepared with gold chloride, and is splitting into discs which show networks of fine lines. (B. Melland.)

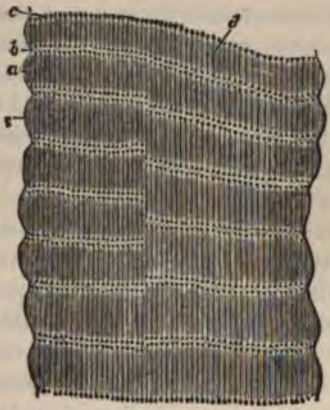

FIG. 69.—Living muscle of Water-beetle highly magnified (E. A. Schäfer). *s*, sarcolemma; *a*, dim stripe; *b*, bright stripe; *c*, row of dots in bright stripe which seem to be the enlarged ends of rod-shaped particles *d*. The transverse filaments connecting these dots are not shown.

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[1] Van Gehuchten, *Anat. Anzeiger*, vol. ii. p. 792 (1887); also in *La cellule* (Louvain), vol. ii. p. 293 (1886); vol. v. (1888), p. 247. In the last-mentioned paper, and also in Quain's *Anatomy*, a bibliography of this subject will be found.

longitudinal and transverse strands quite regularly, as denoted in fig. 68; the interfibrillary substance is the doubly refracting substance.

The longitudinal strands extend throughout the length of the fibre, and the cross strands connect these in the centre of what appears in a resting muscle to be the light stripe (Dobies' line). Under polarised light with crossed nicols the fibrillar network is dark, i.e. because it is singly refracting, the enchylema is bright because it is doubly refracting.

The question arises, how, then, is the ordinary appearance of the alternate striping of a muscular fibre produced? No doubt this is an optical effect; an oil globule examined in water appears surrounded with a halo of light; a row of such globules would have a bright line on each side of it; so the cross strands of the network which are not of equal thickness, but have minute thickenings at the points where the fibres join together, produce a similar effect, and thus the enchylema on each side of the transverse strands appears bright in comparison with the rest. On contraction the longitudinal strands become shorter, and the cross strands thicker, and the granules in the cross strands larger; hence the cross strands now appear dark, while the rest of the enchylema appears bright in comparison. This is the explanation of the apparent interchange in position of the light and dark striæ on muscular contraction.

This view of muscular structure and contraction is much simpler than the complicated theories formerly advanced; it brings muscular fibres into the general category of cells, and shows that the optical appearances that vary with the focus of the microscope and the state of contraction of the muscle may all be explained easily on the supposition that the fibrillar network has different optical properties from those of the interfibrillar stroma or enchylema which it pervades.

Though by means of the microscope and polariscope it is thus possible to distinguish the existence of two substances, it is not possible to say whether the changes that occur on contraction are active in both substances, or whether the movements of one, e.g. the isotropous material, are active, and those of the anisotropous material are merely passive, or *vice versa*.[1] It is also not possible to say whether there is a transference of any material, e.g. water from one to the other during contraction. One must, however, be very careful to recognise that both substances are merely semi-fluid; there is no justification for supposing that anything in the nature of a solid, firm network pervades the interior of the fibre; the nematoid worm seen by Kühne in the interior of a fibre had no difficulty in progressing in any direction.

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[1] Rollett (*Arch. f. mikr. Anat.* 1888, p. 233), for instance, regards the anisotropous material as the actively contractile part of the muscle, and looks upon the network stainable by gold chloride, which Marshall and Melland consider to be the actively contractile part, as merely interfibrillar material. Haycraft's theory (*Brit. Med. Journ.* ii. [1890] 405) comes into the same category as Rollett's.

In macroscopic as opposed to microscopic chemistry, it is not possible to say whether any one of the constituents of the muscle-plasma corresponds to one or other of the two optically different substances; but by microchemical methods, the question of the chemical composition of these substances has been the subject of research by several investigators. Brücke[1] was the first to determine that muscle does contain two substances which act differently on polarised light; and he assumed that the doubly refracting substance is made up of innumerable positive doubly refracting particles with the properties of uniaxal crystals, to which he gave the name *disdiaclasts.* Ebner considers that the action of polarised light does not prove that the two substances are chemically different, but merely that there are alternating differences in the elastic tension of different parts of the muscle-substance. Others, again, have supposed that the only difference chemically is a difference of water, the enchylema being the more watery of the two substances; while others, again, have endeavoured to determine what constituent it is in the muscle-substance that produces the double refraction. Thus O. Nasse[2] believes that the anisotropous (doubly refracting) substance is myosin; the precipitate produced by adding alcohol to a saline solution of myosin is thready like fibrin, and, like fibrin, these threads refract light doubly. C. Schipiloff and A. Danilewsky[3] find that the more myosin is dissolved out of muscular fibres by saline solution, the less do they refract light doubly; they consider that the double refraction of muscle is chiefly produced by myosin, but also partly by lecithin. Myosin is converted into acid-albumin or syntonin very easily by the action of hydrochloric acid; Danilewsky[4] speaks of the substance formed in this way as HCl-myosin; by neutralising the acid he states that he once more obtains true myosin; but this is somewhat contradicted by the fact that it no longer doubly refracts light. He has, therefore, advanced the hypothesis, that myosin may exist in one of two conditions—doubly refracting myosin and singly refracting myosin. The doubly refracting myosin he also calls crystalloid myosin; this is the form in which myosin exists in the muscle, and is apparently the same thing as Brücke's disdiaclasts.[5]

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[1] Stricker's *Handbuch*, chap. vi. p. 170.

[2] O. Nasse, *Zur Anat. u. Physiol. der quergestreiften Muskelsubstanz*, Leipzig, 1882 (Vogel); *Biolog. Centralbl.* 1882, ii. No. 10.

[3] Catherine Schipiloff and A. Danilewsky, *Zeitschr. f. physiol. Chem.* v. 349. These observers also consider that the action of acids and gastric juice in splitting up muscular fibres into discs is due to the solution of lecithin, which they consider to be especially abundant in the centre of the light stripe. This, however, does not appear to me to be proved by their experiments.

[4] Danilewsky, *Zeitschr. f. physiol. Chem.* v. 158.

[5] For Bernstein's views on this subject, *see* p. 435.

None of these experiments, however, prove that the isotropous material contains no myosin; they only show that the anisotropous material contains myosin, or the myosin precursors.

The red variety of voluntary muscular fibres.—W. Krause[1] was the first to notice that certain muscles in the rabbit (soleus, semi-tendinosus, crureus, &c.) were redder in colour than the rest. Similar red muscles have since been described in other mammals and in fishes (rays). These fibres differ from ordinary voluntary muscular fibres in having a longer latent period and a slower contraction; they differ histologically in being more distinctly striated longitudinally, in possessing numerous nuclei which are not confined to the sarcolemma, and in the arrangement and size of their capillary blood vessels.

Chemically the only important difference is the existence in the interior of the muscular fibres of a larger quantity of hæmoglobin than is present in the pale muscles.

Cardiac muscular fibres.—These are quadrate cells without sarcolemma, and with one nucleus in the centre of each. They are branched, and the branches of neighbouring fibres are united by cementing substance which is stained brown by silver nitrate, as is the cementing substance between epithelial cells. The fibres show a well-marked longitudinal striation, an imperfect cross striation, and by polarised light a similarly imperfect fibrillar network is seen to be present throughout the enchylema; the latter is doubly refracting, as in voluntary muscle.

Unstriated or plain muscular fibres.—Voluntary muscular fibres are so much altered from the condition of a primitive cell that the resemblances require to be carefully sought for. Cardiac muscular fibre may be regarded as in an intermediate condition of specialisation, while plain muscular fibres have lost very few of the histological characteristics of primitive cells.[2] They are spindle-shaped, or in the blood vessels sometimes have jagged extremities; each possesses a single nucleus, which is rod-shaped, and has the characteristic structure of nuclei. Each possesses a fine sheath; each exhibits faint longitudinal striation; and by appropriate reagents the protoplasm can be shown to consist of an enchylema pervaded by a fibrillary network. The fibrils run in a longitudinal direction within the fibre.

They never show any double refraction either during life or after death; perhaps the anisotropous substance is absent; myosin is, however, present, as we shall see later on.

Perhaps, as Hoppe-Seyler[3] says, the axes of the particles which produce double refraction (Brücke's disdiaclasts) are differently arranged, so that the light passes through their principal axis, and is thus singly refracted. This seems improbable, however, as there is no double refraction in whatever direction the fibres are viewed.

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[1] *Anatomie des Kaninchens*, 1863.

[2] The cement substance between these fibres is stained brown by silver nitrate.

[3] *Physiol. Chemie*, p. 669.

CHEMICAL COMPOSITION OF MUSCLE

A muscle may be considered as composed of two parts, the supporting connective tissue often containing fat in small quantities, and the muscular fibres themselves, each of which again consists of two parts, the sarcolemma and the contractile substance which it encloses.

The connective tissue of muscle resembles connective tissue elsewhere; the gelatin and fat obtained in analyses of muscles are derived from this tissue. The sarcolemma is a substance which resembles elastin very closely in its solubilities.[1]

The contractile substance is during life of semi-liquid consistency, and contains a large percentage of proteids and smaller quantities of various extractives and inorganic salts. By the use of a press, this substance can be squeezed out of perfectly fresh muscles, and it is then called the muscle-plasma. After death muscle-plasma like blood-plasma coagulates (thus causing the stiffening known as *rigor mortis*). The solid clot corresponding to the fibrin of blood-plasma is called myosin, and the liquid residue is called the muscle-serum.

Living muscle has in the resting condition an alkaline reaction; after extreme activity, and after death, the reaction becomes acid; this is due to the formation of sarco-lactic acid. There are other changes that occur on contraction and on death of muscle, but the details will be considered later.

In round numbers, muscle consists of—

75	per cent.	water.	21	per cent.	proteids and albuminoids.
25	„	solids.	4	„	fat, extractives, and salts.

The following tables give more accurate data concerning the composition of muscle:—

The percentage of water varies somewhat in different animals:—[2]

Man	72–74	per cent.	Birds . . .	70–76	per cent.
Ox	77	„	Amphibians . .	76–80	„
Pig	78	„	Fishes . . .	79–82	„
Cat	75	„	Crab . . .	85	„
Fox	74	„	Pecten (a mollusc)	79–80	„

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[1] Recent experiments on the solubilities of elastin, sarcolemma, and basement membranes have been made by Ewald (*Zeit. Biol.* xxvi. 1).

[2] Schlossberger, *Chemie der Gewebe*, Leipzig u. Heidelberg, 1856, p. 169; Gorup-Besanez, *Lehrbuch*, 1878, p. 676; Hoppe-Seyler, *Physiol. Chemie*, p. 686.

In young animals the percentage of water is greater in the muscles than in those which are fully grown; general inanition also increases the amount of water in the muscles.

Human muscle (Pectoralis major) gave the following average results:—

Water	73·5
Solids	26·5

Proteids, including sarcolemma, proteids of connective tissue, vessels, and pigments	18·02
Gelatin from the connective tissue of muscle	1·99
Fat	3·27
Extractives, creatine, lactic acid, glycogen, &c.	0·22
Inorganic salts	3·12

Chittenden[1] made a similar analysis of the plain muscular fibres of Pecten irradians; he found—

Water	79·60	to	80·25
Solids	20·40	„	19·75
Proteids	15·68	„	15·04
Glycogen	2·43	„	1·98
Glycocine	0·71	„	0·39
Ethereal extractives	0·33	„	0·24
Inorganic salts	1·26	„	1·22

The proteids of muscle will be dealt with in the succeeding sections relating to muscle-plasma, and the phenomena of *rigor mortis*; subsequent sections will deal with the pigments, the extractives, the inorganic salts, and the gases of muscle.

The Muscle-plasma and the Muscle-serum

Kühne[2] was the first to obtain muscle-plasma and to study its reactions; he used frog's muscle. A stream of 0·5 per cent. salt solution injected through the aorta washed out the blood from the muscles; these were then removed, cut into small pieces, kneaded with salt solution at 0° C. (to rid them of lymph), frozen, sliced with cooled knives, pounded in cooled mortars, and then subjected to strong pressure at the atmospheric temperature. The muscle thaws at 0° C. and the liquid pressed out has therefore this temperature; this is filtered and the filtrate is muscle-plasma. It has a syrupy consistency, and a faintly alkaline reaction. At the temperature of the air it soon clots, and the clot of myosin contracts, but not to so great an extent as fibrin does; the liquid squeezed out by the contraction of the myosin is called the muscle-serum. Coagulation begins at the points of contact, and is hastened by agitation and by a temperature of about 40° C. The muscle-serum contains, according to Kühne, three proteids: (1) a proteid which coagulates on heating to 45° C.; (2) an alkali-albumin;[1] (3) an albumin probably identical with serum-albumin. Besides proteids, muscle-serum contains the extractives and salts.

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[1] *Ann. Chem. Pharm.* clxxviii. 260.

[2] Kühne, *Lehrbuch der physiol. Chemie*, p. 272; *Untersuchungen über das Protoplasma*, Leipzig, 1864.

Since then it has been shown[2] that the same facts are true for mammalian voluntary muscle; not only does cold prevent the coagulation of muscle-plasma, but, as in the case of blood-plasma, admixture with solutions of neutral salts (sodium chloride, magnesium sulphate, sodium sulphate, &c.) also prevents it from undergoing coagulation. Addition of water to the salted muscle-plasma so obtained brings about coagulation; that is to say, the concentrated saline solution prevents coagulation, but a dilute saline solution has not this inhibitory influence; this again is exactly similar to what occurs with blood-plasma. The coagulation of the diluted salted muscle-plasma occurs readily at temperatures between 30° and 40°, more slowly at lower temperatures, and not at all at 0° C. Simultaneously with the production of a clot of myosin, sarco-lactic acid is formed. The similarity between the clotting of blood and of muscle is so great that a similar method of formation is suggested; just as fibrin is formed from fibrinogen by the action of fibrin-ferment, so may myosin be formed from a precursor (myosinogen) by the action of a similar ferment. This supposition was found to be fully confirmed when put to the test of experiment. Saline extracts of muscle which has undergone *rigor mortis* resemble salted muscle-plasma very closely; after dilution they undergo coagulation, which can be described as a re-coagulation of the redissolved myosin; the process resembles in all particulars the coagulation of the muscle-plasma which in the first instance leads to the formation of myosin. A saline extract of rigid muscle is, however, acid, and its acidity is increased on re-coagulation.

The properties of the muscle-clot (*myosin*).—Myosin may be prepared by allowing muscle-plasma to clot, or on dilution of saline extracts of either absolutely fresh frozen muscle, or of muscle which has undergone rigor. Ammonium chloride solution extracts myosin from muscle in greater quantity than other salts, and then the myosin may be precipitated in a gelatinous form by dialysing away the salt (Kühne and Chittenden[1]). Elementary analysis of the myosin so obtained gives the following results:[2] C, 52·79; H, 7·12; N, 16·86; S, 1·26; O, 22·97.

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[1] This is probably what we shall call later myoglobulin. The various tissue-caseins or alkali-albumins that have been described in tissues are no doubt all globulins.

[2] Halliburton, 'On Muscle-plasma,' *Journ. of Physiology*, viii. 133–202.

Myosin is precipitated by dropping a saline solution of it into excess of distilled water. It is readily soluble in 5 to 10 per cent. solutions of sodium chloride and other neutral salts; it is precipitated from its solutions by saturation with sodium chloride, magnesium sulphate, and ammonium sulphate. These properties clearly place myosin among the globulins. It is very readily soluble in weak hydrochloric acid, forming syntonin or acid-albumin. It is readily digested by gastric juice, forming peptones, the intermediate products being called myosinoses; it is still more readily dissolved by pancreatic juice. Myosin, like fibrin, decomposes peroxide of hydrogen. If one takes a perfectly neutral solution of myosin in 5 per cent. sodium chloride solution and then dilutes this with two or three times its volume of water, a formation of a coagulum of myosin takes place, as in the case of muscle-plasma; that is to say, there is first a jellying throughout the liquid; the coagulum subsequently contracts and squeezes out a clear liquid; this occurs most readily at the body temperature, and the addition of myosin ferment hastens the formation of the clot. Thus it appears to be a true ferment coagulation or re-coagulation;[3] this view is supported by the fact that the previously neutral liquid is now acid from the presence of sarco-lactic acid. The liquid squeezed out by the contraction of the clot is free from proteid.

We have seen the similarities between the formation of fibrin and that of myosin; the differences may be summarised as follows:—

(1) Fibrin dissolves with difficulty in saline solutions (e.g. 5-10 per cent. sodium chloride); the dissolved fibrin has not the properties of fibrinogen, and cannot be made to yield fibrin again. Myosin is readily soluble in such saline solutions; the dissolved myosin has the properties of myosinogen, and on suitable treatment can be reconverted into myosin.

(2) The formation of myosin from myosinogen is accompanied by the development of acid, whereas that of fibrin from fibrinogen is not, so far as we know.

(3) The formation of myosin from myosinogen is not accompanied by the formation of another globulin, whereas that of fibrin from fibrinogen is (*see* p. 235).

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[1] Myosin and Myosinoses, *Zeit. Biol.* vol. xxv. 358.

[2] *Ibid.*

[3] Chittenden also regards this as a re-coagulation, *Studies from the Lab. of Physiol. Chem. Yale Univ.* vol. iii. 1889, p. 116.

Myosin when very thoroughly washed with water, or dialysed free from salts, becomes very insoluble both in saline solutions and in 1 per cent. hydrochloric acid. Danilewsky[1] considers that the HCl-myosin (as he terms syntonin) depends for its formation on the presence of a small quantity of calcium salts.

The formation of acid during coagulation.—When muscle contracts vigorously it becomes acid; when it undergoes *rigor mortis* it becomes acid; when muscle-plasma clots or myosin is formed from myosinogen, acid is developed; and when saline extracts of rigid muscle undergo re-coagulation, they become more acid than they were previously.

Numerous researches have shown that this acid is lactic acid (Berzelius,[2] Du Bois Reymond,[3] Kühne,[4] Heidenhain[5]). Weyl and Seitler[6] consider, however, that in the earlier stages of muscular activity the acid reaction may be partially due to an acid potassium phosphate produced from the alkaline phosphate by the development of new phosphoric acid from organic phosphorised compounds like lecithin and nuclein in the muscle.

Lactic acid may be identified by the following reaction:—

A solution of dilute ferric perchloride and carbolic acid is made as follows:—

10 c.c. of a 4 per cent. solution of carbolic acid.

20 c.c. of distilled water.

1 drop of the liquor ferri perchloridi of the British Pharmacopœia.

On mixing a solution containing only a mere trace of lactic acid with this violet solution, it is instantly turned yellow (Uffelmann[7]). The test may be more accurately performed as follows: The muscle extract is boiled, filtered, and extracted with an equal volume of ether; the ethereal extract is evaporated to dryness, the residue taken up with water, and then the test tried with this aqueous solution. The test is especially delicate for lactic acid, being given by a solution of it consisting of one part to 10,000 of water.

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[1] *Zeit. physiol. Chem.* v. 158–184.

[2] Berzelius, *Lehrbuch der Chemie*, vi. 557.

[3] Du Bois Reymond, *Gesammelte Abhandl. zur allgemeinen Muskel- und Nerven-Physik*, Leipzig, 1877.

[4] Kühne, *loc. cit.*

[5] Heidenhain, *Mechanische Leistung*, p. 143.

[6] Weyl and Seitler, *Zeit. physiol. Chem.* vi. 557.

[7] J. Uffelmann, *Zeit. f. klin. Med.* viii. 392. Caher and Mering (*Deutsch. Archiv f. klin. Med.* xxxix. 242) have cast doubt on the trustworthiness of this test for lactic acid in the presence of hydrochloric acid as in the stomach. There can, however, be no question of hydrochloric acid in muscle, and it takes larger percentages of hydrochloric acid (more than 0·2 per cent.) to decolourise the test solution.

There are at least three varieties of lactic acid; all have the formula $C_3H_6O_3$; these are:—

(1) Sarco-lactic or para-lactic acid; this is dextro-rotatory.

(2) Ordinary lactic acid, such as is formed during the souring of milk. This differs from (1) in (*a*) the amount of water of crystallisation of its salts (the zinc and calcium salts are those usually prepared), and (*b*) in its solutions having no action on polarised light. Both (1) and (2) have the same chemical constitution, and are called ethidene lactic acid.

(3) Ethene lactic acid.

The lactic acid of muscle is chiefly (1); a small quantity of (3) is also present.

Observers differ, however, as to the origin of lactic acid. O. Nasse[1] believes that it comes from the glycogen present in muscle. Most physiologists, however, are inclined to regard the proteids as its source; this seems to me to be very conclusively shown by the experiments of R. Böhm.[2] Böhm found that the amount of glycogen in putrefaction and in rigor remains unaltered from that in fresh muscle; glycogen, therefore, cannot be the source of the acid. I quote one of Böhm's tables which brings out this point clearly.

Experiment	Fresh muscle		Muscle after rigor	
	Percentage of		Percentage of	
	Glycogen	Lactic acid	Glycogen	Lactic acid
1	0·71	0·22	0·71	0·57
2	0·28	0·16	0·28	0·44
3	0·036	0·35	0·041	0·56

This view of Böhm's is endorsed by Hoppe-Seyler.[3]

The close relationship in point of time between the change in the condition of the proteids and the development of an acid reaction certainly supports the view that lactic acid arises in some way from the proteids. How this precisely takes place will not be known until we are acquainted with the rational formula of proteids. It is here, however, worth noting that Latham,[4] who has a very distinct theory as to the composition of proteids (*see* p. 116), is also by his theory able to denote by a formula the way in which lactic acid arises after the death or during the contraction of a muscle. The theory advanced by Latham is, that albumin is a compound of cyan-alcohols united to a benzene nucleus. The following are Latham's words concerning lactic acid:—

'The lactic acid developed when a muscle contracts or dies is a mixture of two kinds of lactic acid, the more abundant being paralactic acid or ethidene lactic acid $CH_3\ CH\left\{\begin{matrix}OH\\COOH\end{matrix}\right.$, the other, ethene lactic acid $CH_2\ CH_2\left\{\begin{matrix}OH\\COOH\end{matrix}\right.$. Now, by treating ethidene cyan-acohol with acids or alkalies, paralactic acid is obtained.

$$\underset{\text{[ethidene cyan-alcohol]}}{CH_3\ CH\left\{\begin{matrix}OH\\CN\end{matrix}\right.} + 2H_2O = NH_3 + \underset{\text{[paralactic acid]}}{CH_3\ CH\left\{\begin{matrix}OH\\COOH\end{matrix}\right.}$$

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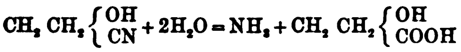
[1] *Zur Anat. und Physiol. der quergestreiften Muskelsubstanz*, Leipzig, 1882.

[2] *Pflüger's Archiv*, xxiii. 44.

[3] *Physiol. Chemie*, pp. 666, 667. *See* also Molinari, *Chem. Centr.* ii. 1889, p. 372.

[4] *British Medical Journal*, vol. i. 1886, p. 630.

'By treating ethene cyan-alcohol in the same way, ethene lactic acid is obtained.

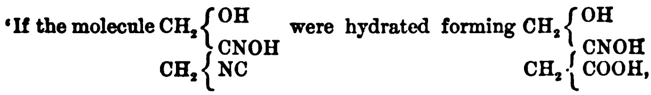


$$CH_2\,CH_2\left\{\begin{array}{l}OH\\CN\end{array}\right. + 2H_2O = NH_3 + CH_2\,CH_2\left\{\begin{array}{l}OH\\COOH\end{array}\right.$$

[ethene cyan-alcohol] [ethene lactic acid]'

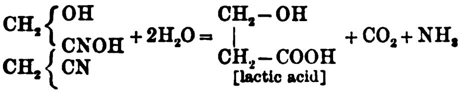
Later on,[1] Dr. Latham says :—

'There is another way in which the molecules may split up, which is interesting, as it appears to explain the formation of lactic acid and carbonic anhydride when a muscle contracts or dies.



'If the molecule $\begin{array}{l}CH_2\left\{\begin{array}{l}OH\\CNOH\end{array}\right.\\CH_2\left\{NC\right.\end{array}$ were hydrated forming $\begin{array}{l}CH_2\left\{\begin{array}{l}OH\\CNOH\end{array}\right.\\CH_2\left\{COOH,\right.\end{array}$

and the portion CNOH then detached, the latter would be at once decomposed into CO_2 and NH_3, and we should have lactic acid left. This is shown in the following formula :—



$$\begin{array}{l}CH_2\left\{\begin{array}{l}OH\\CNOH\end{array}\right.\\CH_2\left\{CN\right.\end{array} + 2H_2O = \begin{array}{l}CH_2-OH\\ |\\ CH_2-COOH\end{array} + CO_2 + NH_3$$

[Two molecules of the lowest term of the cyan-alcohol series] [lactic acid]

'The ammonia (NH_3) would not be set free, but would unite with some other cyan-alcohol higher in the series to form a cyanamide.'

In connection with the presence of lactic acid in muscle after death, Catherine Schipiloff[2] has formed an ingenious theory of *rigor mortis*. She finds that myosin can be precipitated from its saline solutions by weak acids without change, and is soluble in excess; the same occurs in the body; by injecting small quantities of lactic or hydrochloric acids (0·1 to 0·25 per cent.) into the vessels of a recently killed frog, the muscles become rigid; this passes off by injecting stronger acid 0·3 to 0·5 per cent. If such stronger acids be injected in the first instance, no rigor, or rigor which is only momentary, occurs. After rigor, muscle contains more acid than either during rigor or before it sets in. The conclusion is therefore drawn that rigor is due to the post-mortem development of acid, and its passing off is due to the development of more acid, which redissolves the precipitated myosin.

If one, however, examines the reasoning which results in the above conclusion the premisses will be found not to bear it out. Because myosin can be precipitated and redissolved by acid when in solution, or after death in the muscles, it does not necessarily follow that this is the cause of rigor in the normal course of things, even if the amount of lactic acid is increased after rigor; for the increase in lactic acid may not only be the cause of rigor, it may also be the result, or merely a concomitant of the coagulation of muscle. I myself believe it to be a concomitant merely of the conversion of myosinogen into myosin, and that both are due to the same cause—a ferment action. Moreover, the theory of C. Schipiloff seems to involve that lactic acid is formed from something other than proteids, presumably glycogen. In addition to this no syntonin is discoverable in rigid muscle.

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[1] *Loc. cit.* 634.

[2] *Centralblatt f. d. Med. Wissensch.* 1882, p. 291.

The ferments of muscle.—The myosin-ferment may be prepared from muscle in the same way as fibrin-ferment is from blood, and the following five propositions state succinctly the chief facts that are known with regard to the ferment:—

1. By keeping muscle under alcohol for some months, most of the proteids are coagulated. Water will, however, extract from the alcoholic precipitate a proteid which has the characters of an albumose.

2. This solution has the properties of a ferment in causing the coagulation of muscle-plasma; it may be that the ferment is in very close combination with the albumose.

3. This myosin-ferment, as it may be termed, does not hasten the coagulation of blood-plasma; nor does fibrin-ferment hasten the coagulation of muscle-plasma; the two are not therefore identical.

4. The juice expressed from fresh muscle, however, hastens very markedly the coagulation of salted blood-plasma. This is not due to its containing fibrin-ferment, but it is due to the proteid substance myosinogen, which enters into the condition of a heat-coagulum at 56° C. Fibrin-ferment is absent, or only present in exceedingly small quantities.[1]

5. The activity of fibrin-ferment is destroyed at 75°–80° C.; the activity of myosin-ferment is not destroyed till the temperature of 100° C. is reached.

It is necessary to allude to the existence of yet other ferments which have been shown to exist in muscle. Brücke has shown that muscle, in common with most of the tissues of the body, contains a small quantity of pepsin. O. Nasse[2] showed that the muscle-juice also contains an amylolytic ferment, which he supposes to act in the transformation of glycogen into sugar after death. I have made a few experiments on this subject and can fully confirm Nasse's statement of the existence of this ferment; a watery extract of the dried alcoholic precipitate of muscle changes glycogen into a reducing sugar; it will also act upon starch in a similar way, and in both cases an intermediate product of the nature of dextrin is formed. The action on starch is, however, slow; at the temperature of 40° C. sugar is not discoverable by Fehling's test till after the ferment has acted upon it for five or six hours.

Proteids of the muscle-plasma.—By fractional heat-coagulation, five proteids can be separated in muscle-plasma; four of these are precipitable by various temperatures, and one of the nature of an albumose is not precipitable by heat. In the muscle-serum three proteids only are found, two having gone to form the muscle-clot.

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[1] E. Grubert ('Ein Beitrag zur Physiologie des Muskels,' *Inaug. Diss.* Dorpat, 1883), J. Klemptner (*Inaug. Diss.* Dorpat, 1883), and E. Kügler (*Inaug. Diss.* Dorpat, 1883) have supposed that fibrin-ferment is really present in muscle. This question will be found fully discussed in my paper on 'Muscle-plasma,' *Journ. Physiol.* viii. 169–182.

[2] *Loc. cit.*

This may be represented in a tabular form as follows :—

Proteids of the muscle-plasma	Proteid precipitated by heat at 47° C.	Proteids which go to form the muscle-clot.
	„ „ „ 56°	
	„ „ „ 63°	Proteids of the muscle-serum.
	„ „ „ 73°	
	„ not „ „ albumose	

No substance of the nature of alkali-albumin or muscle-casein is present. Peptones are also absent.[1]

The next table gives the names applied to these various proteids, and the influence of neutral salts upon them in causing precipitation.

Proteid precipitated by heat at	Name	Saturation with sodium chloride or magnesium sulphate	
47° C.	Paramyosinogen[2]	causes precipitation	Globulins
56°	Myosinogen	causes precipitation	
63°	Myoglobulin	causes precipitation	
73°	Albumin	does not cause precipitation	
Proteid not precipitated by heat	Myoalbumose	does not cause precipitation	

The following is a scheme for the separation of these proteids from one another :—

Salted Muscle-plasma.—Dilute to six times its volume, and expose this to a temperature of 35° C. for one or two hours. It separates into clot, and salted muscle-serum. Filter.

- *Clot* : consists of myosin. Wash with water, redissolve in 5 per cent. magnesium sulphate solution. Heat to 47° ; a precipitate is produced. Filter.
  - *Precipitate* : consists of paramyosinogen
  - *Filtrate* : contains myosinogen, which is precipitated at 56° C.
- *Salted muscle-serum* : contains myoglobulin, albumin, and myoalbumose. Saturate with magnesium sulphate or sodium chloride ; a precipitate is produced. Filter.
  - *Precipitate* : consists of myoglobulin
  - *Filtrate* : contains albumin and myoalbumose. Heat to 73° ; a precipitate is produced. Filter.
    - *Precipitate* : consists of albumin
    - *Filtrate* : contains myoalbumose

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[1] Fischel (*Zeit. physiol. Chem.* x. 14) and M. Muira (*Virchow's Archiv*, ci. 316) have described peptone in muscle; perhaps they have mistaken the albumose for peptone.

[2] Termed musculin by Hammarsten, *Fysiologisk Kemi.* Upsala, 1889, p. 218.

These proteids may also be separated from one another by the use of different quantities of neutral salts. This will be seen in the following summary of the properties of each proteid. Of the two proteids that go to form the muscle-clot, one only is essential; the other, named paramyosinogen, is apparently carried down with it more or less mechanically. This proteid was found in frog's muscle by Kühne, and in the muscles of various animals by Demant.[1] Demant estimated the amount of it present in both voluntary and involuntary muscles; he often found the merest traces, but the general average was 0·5 per cent. He, however, incorrectly speaks of it as an albumin; it is, as we have seen, a globulin.

Paramyosinogen	*Myosinogen*
1. It coagulates at the temperature of 47° C. The precipitate is flocculent.	1. It coagulates at the temperature of 56° C. The precipitate is sticky.
2. It is partially precipitated in a magnesium sulphate solution of the strength of 37 per cent.	2. It is partially precipitated in a magnesium sulphate solution of the strength of 60 per cent.
3. It is precipitated completely when the strength of the magnesium sulphate solution is 50 per cent.	3. It is precipitated completely when the strength of the magnesium sulphate solution is 94 per cent.
4. It is partially precipitated in a sodium chloride solution of the strength of 15 per cent.	4. It is partially precipitated in a sodium chloride solution of the strength of 30 per cent.
5. It is precipitated completely when the strength of the sodium chloride solution is 26 per cent.	5. It is completely precipitated when the strength of the sodium chloride solution is 36 per cent (i.e. saturated).
6. The precipitate produced by these salts is curdy, and settles in coarse flocculi.	6. The precipitate produced by these salts is a fine precipitate which settles into a semi-gelatinous deposit.
7. It is rendered insoluble in dilute saline solutions, by prolonged washing with saturated saline solutions.	7. It is rendered insoluble in dilute saline solutions, by prolonged washing with saturated saline solutions.
8. It takes part in the formation of the muscle-clot, but does not, by itself, coagulate under the influence of myosin-ferment.	8. It takes an essential part in the formation of the muscle-clot, and coagulates under the influence of the myosin-ferment.

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[1] Demant, *Zeit. physiol. Chem.* iii. 241; iv. 386.

9. It is not precipitated from its saline solutions by acetic acid.	9. Acetic acid gives when added to a solution of this proteid a characteristic stringy precipitate.
10. It has no power in hastening the formation of fibrin in blood-plasma.	10. It has a very marked power of hastening the formation of fibrin in blood-plasma.

Myoglobulin resembles serum-globulin very closely in its properties, the most marked difference being in its coagulation temperature, which is 63° C., while that of serum-globulin is 75° C. It is not precipitated by sodium chloride or magnesium sulphate from its solutions until they are completely saturated with that salt.

Muscle-albumin is apparently identical with serum-albumin, and it coagulates at a temperature of 73° C.

Myoalbumose (or myoproteose, as it might more properly be called) is closely connected to, or identical with the myosin-ferment ; it gives the reactions of the deutero-albumose of Kühne and Chittenden (*see* DIGESTION).

These five proteids (together with hæmoglobin in the case of the red muscles) constitute the proteids of the muscle-plasma.

Rigor mortis, which is due to the coagulation of the muscle-plasma within the sarcolemmas of the muscular fibres, produces a hardening of the muscle and a lessening of its extensibility and elasticity. A dead muscle is negatively electrical to a living one.

The onset of rigidity varies in man from ten minutes to seven hours; after a time it passes off. Its duration is variable from one to six days. Great muscular activity before death causes rapid and intense rigidity, and the earlier it occurs the shorter time does it last. Various drugs favour the early onset of rigidity, e.g. quinine, caffeine, digitaline, veratrine, hydrocyanic acid, ether, chloroform, &c.

The cause of the disappearance of rigor mortis after its onset is still a matter of doubt. The usual explanation of the phenomenon is that it is due to putrefaction ; still there are many cases in which rigor passes off before putrefaction sets in, and other cases in which rigor persists after the onset of putrefaction. Cossar Ewart[1] has shown that in fishes there is a persistence of rigidity if putrefaction be prevented and bacteria excluded by a weak solution of mercuric chloride. I am, however, of opinion that putrefaction will not explain all the facts, but am inclined to think that more probably the disappearance of rigor is due to the action of an unorganised ferment. We have already seen that muscle contains pepsin, and that it turns acid when it dies. If dead muscle be kept at about the temperature of the body for a few hours after death, rigor passes off rapidly, and the muscle is found to contain, not only its normal proteids, but also the products of gastric digestion; proteoses[1] and peptone are found in abundance. Muscle has, in other words, undergone a process of self-digestion. In ordinary circumstances self-digestion does not go so far as this, but only results in the breaking down of myosin into myosinogen, and this would be quite sufficient to cause softening of the muscle. We have already seen how easily the change of myosinogen into myosin, and *vice versâ*, is brought about. The action of pepsin in producing the change is quite analogous to what we have already seen in the case of fibrin; before the formation of albumoses and peptone sets in, the fibrin is decomposed into soluble globulins (*see* p. 233).

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[1] *Proc. Physiol. Society*, 1887, p. xxv.

This view of the cause of the disappearance of rigor must not, however, be taken as by any means fully proved. Observations are still necessary with regard to the relation between the rapidity of the disappearance of rigor and the amount of pepsin in the tissues, which probably varies with the condition of the alimentary canal.

Rigor mortis in involuntary muscle.—So far as can be stated from actual work done, which is scanty, on this subject, the phenomena of *rigor mortis* in some involuntary muscles are much the same as in the voluntary muscle. The heart becomes rapidly rigid, and simultaneously acid from the formation of sarco-lactic acid. Both paramyosinogen and myosinogen are present in the muscle-cells of the heart, and myosin is the result of coagulation.

In the stomach and uterus, rigor has been observed, but in other forms of plain muscle it is difficult to recognise and has never been satisfactorily observed. Myosin, i.e. a globulin entering into the condition of a heat-coagulum at 56° C. has been obtained from all kinds of unstriped muscle. Kossel[2] examined the proteids present in a muscular tumour of the uterus, and found the proteid which coagulates at 45° C. (paramyosinogen) absent.

The reaction of unstriped muscle is normally alkaline.[3] Lehmann[4] found small quantities of lactic acid in the muscular substance of the stomach after death. There is, however, no marked change in reaction after death as in striated muscle. Du Bois Reymond[5] observed that the muscles of the stomach and intestines of the bird were, after death, still alkaline.

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[1] The albumose or proteose already described as myoalbumose does not appear to be a product of digestion, but exists normally in muscle-plasma.

[2] Quoted by Hoppe-Seyler, *Physiol. Chemie*, p. 669.

[3] Bernstein (Kühne's *Lehrbuch*, p. 332) found the actively contracting muscles of the anodon acid.

[4] *Lehrbuch*, iii. 73.

[5] *Monatsberichte d. Berl. Akad. d. Wissensch.* 1859, p. 312.

We have thus seen that there is a certain amount of knowledge regarding the changes in the proteids that occur when muscle dies; but whether any changes take place in the proteids during muscular contraction is quite unknown.

The Pigments of Muscles

Hæmoglobin.—This is contained in the muscle-plasma of the red muscles of rodents and other animals. Kühne showed that hæmin-crystals can be obtained from it. The pale muscles also often contain small quantities of hæmoglobin.

In certain gastropod molluscs (Limnæus, Paludina) hæmoglobin is absent from the blood, but is present in the muscular fibres of the pharyngeal wall (Lankester).

When coagulation of the muscle-plasma takes place, the hæmoglobin plays no part in the formation of the muscle-clot, but passes into the muscle-serum.

Myohæmatin.—This is one of the most important of a group of pigments discovered by MacMunn, and named by him the histohæmatins.[1] These pigments have a wide distribution in the animal kingdom, and often occur in those animals which possess no hæmoglobin. They have not, however, been separated in a pure condition, but have only been observed by means of the spectroscope. In so far as conclusions can be drawn from spectroscopic observations, these substances appear to exist in two conditions analogous to those of hæmoglobin and oxyhæmoglobin; that is to say, by means of reducing agents and oxidising agents, different spectroscopic appearances are produced; in the reduced condition they show well-marked bands, and in the oxygenated condition the bands are faint or fade altogether from view. MacMunn considers that these pigments are respiratory pigments, holding the oxygen brought to the tissue by the blood until it is required by the tissue. The spectrum of these substances is somewhat like that of hæmochromogen.[2]

Myohæmatin, the most widespread of the histohæmatins, occurs in the muscles of insects, spiders, crustaceans, molluscs, fishes, amphibians, reptiles, birds, and mammals; in man it has been found in the heart and in the voluntary muscles. The heart-muscles of the pigeon contain the pigment in great abundance, there being little or no hæmoglobin present. If a portion of such a muscle be rendered transparent by glycerine, and then by means of a compressorium it be squeezed out to a great degree of thinness, the bands are readily visible (*see* fig. 70). The bands are four in number.

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[1] MacMunn, 'Myohæmatin and the Histohæmatins,' *Phil. Trans. of Royal Society*, Part I. 1886; *Journ. of Physiol.* viii. No. 2.

[2] Hoppe-Seyler (*Zeit. physiol. Chem.* xiii.) believes myohæmatin is hæmochromogen.

By artificial gastric digestion the bands are altered, and this constitutes what MacMunn calls modified myohæmatin. Modified myohæmatin can be obtained in solution by covering the blood-free muscles with ether for some days. A yellow lipochrome derived from the fat between the muscular fibres[1] passes into solution, and below this floats a red juice which shows the two bands of modified myohæmatin (fig. 70, spectrum 2); these resemble those of hæmochromogen, but are placed rather nearer the violet.

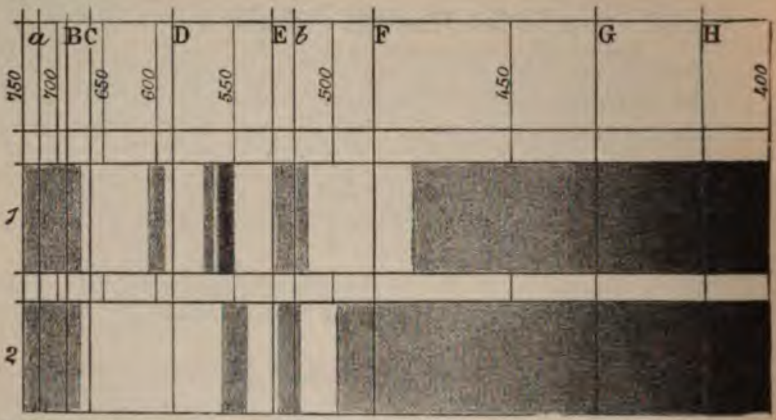

FIG. 70.—1, Absorption spectrum of myohæmatin. First band, λ 613-600; second band, λ 569-563; third band, λ 556-550, this is the best marked band; fourth band, an ill-defined shading over the *b* line. 2, Absorption spectrum of modified myohæmatin. First band, λ 554·5–548·5; second band, λ 524·5–519.

The Extractives of Muscle

The extractives of muscle may be divided into two sets:—

A. *Nitrogenous*
- Creatine
- Creatinine
- Xanthine
- Hypoxanthine
- Carnine
- Uric acid
- Urea
- Taurine
- Inosinic acid

B. *Non-nitrogenous*
- Fats
- Glycogen
- Inosite
- Fermentable sugar
- Lactic acids

The method of preparation and chief properties of each of these substances will now be taken up *seriatim*.

Creatine.—If an aqueous infusion of meat be made, and the proteids precipitated by boiling and filtering off the coagulum so formed, the extractives and salts remain in solution. Beef tea is a liquid which contains little more than extractives and salts; Liebig's and other commercial meat extracts are virtually the solid residues obtained on evaporating aqueous infusions (from which the proteids have been separated) to dryness.

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[1] Halliburton, *Journ. of Physiol.* vii. 325.

The following methods of preparation may be adopted:—

1. To an aqueous extract of meat (*minus* its proteids) add baryta to precipitate the phosphates; filter; remove excess of baryta from the filtrate by a stream of carbonic acid; filter off the barium carbonate; and evaporate the filtrate on the water-bath to the consistency of a thick syrup. Set it aside to cool, and in a few days crystalline deposits of creatine will be found at the bottom of the vessel. These are washed with alcohol and dissolved in hot water. On concentrating the aqueous solution crystals once more separate out, which may be still further purified by recrystallisation (Liebig[1]).

2. The aqueous extract is precipitated with acetate of lead; filtered; the filtrate freed from excess of lead by a stream of sulphuretted hydrogen, and then filtered and evaporated till crystals appear, which may be purified by recrystallisation (Neubauer[2]). Städeler[3] uses an alcoholic instead of an aqueous extract of muscle.

3. Muscle is finely chopped and allowed to stand under ether; a strongly acid, watery fluid in a day or two separates out; this is red owing to the presence of myohæmatin; the ether floats above this watery liquid. On evaporating the latter, crystals of creatine separate out, and may be purified by recrystallisation as before (MacMunn[4]).

Creatine has the formula $C_4H_9N_3O_2$; this unites with one molecule of water of crystallisation to form transparent, colourless, monoclinic prisms (fig. 34, p. 84). The crystals lose their water of crystallisation at 100° C. They are soluble in water, especially in hot water, and almost insoluble in absolute alcohol and in ether, sparingly soluble in rectified spirit.

Creatine forms crystalline compounds with the mineral acids, and with mercury ($C_4H_7HgN_3O_2$).

When creatine is treated with various reagents it undergoes a number of different decompositions. The most important of these are the two following, as it is probable that similar changes occur in the body:—

(*a*) *Conversion into creatinine.*—When creatine is heated with dilute mineral acids, or for several days with water, it loses a molecule of water and creatinine is formed :—

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[1] *Ann. d. Chem. u. Pharm.* lxii. 257.

[2] *Ibid.* cxix. 27.

[3] *J. f. prakt. Chem.* lxxii. 256.

[4] *Journ. of Physiol.* viii. 58.

$$\underset{\text{[creatine]}}{C_4H_9N_3O_2} - H_2O = \underset{\text{[creatinine]}}{C_4H_7N_3O}$$

A similar change occurring in the body no doubt gives rise to the creatinine occurring in the urine.

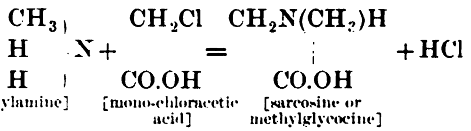
(*b*) *Conversion into sarcosine and urea.*—Creatine seems to replace urea in muscular tissue;[1] the theory that it is a stage in the formation of urea has been advanced because it can be made to yield urea in the laboratory; its molecule, in fact, contains the cyanamide radicle ($CN.NH_2$), which *plus* a molecule of water is equal to urea (CON_2H_4). (*See* further under Urine.)

When creatine is boiled with baryta water, the following equation represents the change that occurs :—

$$\underset{\text{[creatine]}}{C_4H_9N_3O_2} + H_2O = \underset{\text{[urea]}}{CON_2H_4} + \underset{\text{[sarcosine]}}{C_3H_7NO_2}$$

Synthesis of creatine.—Creatine has been made synthetically, and the following, which is the method adopted, will show what is the constitution of its molecule.

When methylamine and monochloracetic acid are brought together, the following reaction occurs :—



$$\underset{\text{[methylamine]}}{\left.\begin{matrix}CH_3\\H\\H\end{matrix}\right\}N} + \underset{\substack{\text{[mono-chloracetic}\\\text{acid]}}}{\begin{matrix}CH_2Cl\\|\\CO.OH\end{matrix}} = \underset{\substack{\text{[sarcosine or}\\\text{methylglycocine]}}}{\begin{matrix}CH_2N(CH_3)H\\|\\CO.OH\end{matrix}} + HCl$$

That is to say, sarcosine and hydrochloric acid are formed. Sarcosine is also called methylglycocine, i.e. glycocine (amido-acetic acid), in which one H is replaced by methyl (CH_3). On heating sarcosine and cyanamide together, creatine is formed according to the following equation :—

$$\underset{\text{[sarcosine]}}{C_3H_7NO_2} + \underset{\text{[cyanamide]}}{CN.NH_2} = \underset{\text{[creatine]}}{C_4H_9N_3O_2}$$

Quantity of creatine in muscles.—According to Voit[3] the quantity of creatine is fairly constant in the voluntary muscles; the quantity is found to vary from 0·2 to 0·3 per cent. in different animals. This quantity increases during starvation (Demant[4]). Cardiac muscle contains less creatine than the voluntary muscles (Voit); the same is true for unstriated muscle (Lehmann[1]).

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[1] Creatine is also found sparingly in nervous tissue.

[2] Creatine is thus methyl-guanidine-acetic acid (Baumann, *Ann. Chem. Pharm.* clxvii. 77).

[3] *Zeit. f. Biologie*, iv. 77. Subsequent analyses by Neubauer, Hofmann, Creite, and many others have confirmed Voit's analyses. For references *see* Hoppe-Seyler's *Physiol. Chem.* p. 642.

[4] *Zeit. physiol. Chem.* iii. 387.

Creatinine.—Small quantities of this base are found in muscle;[2] it is also a constituent of urine. The strongly alkaline reaction of creatinine is said by Salkowski in a recent article[3] to be greatly due to adherent alkaline salts. The compound of creatinine with zinc chloride $(C_4H_7N_3O)_2ZnCl_2$ has a characteristic crystalline form, and is used as a test for this substance. The statements made as to the relative quantities of creatine and creatinine during the rest and activity of muscle are contradictory; Voit[4] states that creatine is diminished during activity; Sarokin[5] that creatinine increases during tetanus; and Nawrocki[6] contradicts these statements. The more recent observations by Monari[7] however confirm the original statement of Sarokin; on fatigue of muscle, creatinine is produced from the creatine, together with a small quantity of another substance called xanthocreatinine $C_5H_{10}N_4O$.

Hypoxanthine, Xanthine, and Uric Acid are found in muscles in small quantities.

The formulæ of these three substances denote their close relationship to one another.

Hypoxanthine or Sarcine	$C_5H_4N_4O$
Xanthine	$C_5H_4N_4O_2$
Uric acid	$C_5H_4N_4O_3$

The last-mentioned substance, uric acid, occurs only in the merest traces (Meissner[8]). With regard to xanthine[9] and hypoxanthine,[10] the following is a summary of the chief facts concerning them:—

Preparation.—The mother liquor, from which creatine has been separated, is precipitated with ammonia and silver nitrate: the precipitate is dissolved in nitric acid of specific gravity 1·1. The liquid is cooled and a compound of hypoxanthine and silver nitrate crystallises out. The mother liquor (*a*) is preserved. The silver is removed from the crystals by sulphuretted hydrogen, and the nitrate of hypoxanthine treated with ammonia, and thus crystalline nodules of hypoxanthine are formed. A compound of xanthine and silver nitrate is left in solution in (*a*); it is precipitated by excess of ammonia; the silver is removed by sulphuretted hydrogen, and the base obtained in white amorphous granules by adding ammonia.

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[1] *Lehrbuch*, iii. 73.

[2] This is, however, denied by Neubauer and Nawrocki.

[3] Salkowski, *Zeit. physiol. Chem.* xii. 211.

[4] *Loc. cit.*

[5] *Virchow's Archiv*, xxvii.

[6] *Centralblatt*, 1865, p. 417.

[7] A. Monari, *Gazetta*, xvii. 367.

[8] *Zeit. f. rat. Med.* xxxi. 144.

[9] Found also in urinary calculi (Marcet), in guano (Unger and Phipson, *Chem. Med.* vi. 16), and in urine (Bence Jones, *Quart. J. Chem. Science*, xv. 78; Weiske, *Zeit. f. Biol.* ii. 254).

[10] Found also in spleen (Scherer), in the blood, marrow, and secreting glands.

Xanthine	*Hypoxanthine*
Properties.—It reduces silver salts. It forms compounds with hydrochloric acid (hexagonal plates), nitric acid, &c.; the latter is not rendered purple by ammonia, and so it can be distinguished from uric acid.	It forms compounds with silver, copper, and platinum. On oxidation with nitric acid it yields xanthine. Its nitrate and chlorate are crystalline.
Amount in muscle.—0·0026 (Scherer[1]).	0·022–0·026 (Neubauer[2]). Its amount increases in starvation (Demant).

Carnine.—A crystalline base ($C_7H_8N_4O_3+H_2O$); it was originally found in fairly large quantities (1 per cent.) in American meat extracts (Weidel[3]); it has been since found in the flesh of several animals (frog, alligator, &c.) by Krukenberg and Wagner.[4] It is probable that carnine is one of the intermediate products between the proteid molecule and the substances of the uric acid group which we have just considered.

Urea.—(CON_2H_4) is probably present in small quantities, but is difficult to separate from other nitrogenous bases.

Taurine.—Found in the muscles of the horse, fishes, and molluscs. In fishes Limpricht[5] found 1·06 per cent. of taurin.

Glycocine.—Found to the extent of 0·39–0·71 per cent. in the non-striated muscles of molluscs (Chittenden[6]).

Protic acid (Protsäure).—An acid formed from the decomposition of proteids described by Limpricht in the muscles of fishes. This is an acid of doubtful nature.

Inosinic acid ($C_{10}H_{14}N_4O_{11}$).—First described by Liebig. It is itself amorphous, but forms crystalline salts with the alkaline metals, with barium, and with calcium. It has since been found and estimated (0·005–0·02 per cent.) by Creite.[7]

Lecithin and its decomposition products, such as glycerophosphoric acid, are found in small quantities in muscle, but it is very doubtful whether this substance is a constituent of muscular substance proper; more probably it is derived from the nerve fibres which terminate in the muscle (Hoppe-Seyler[8]).

The next class of extractives are those which are non-nitrogenous; these are glycogen, inosite and other carbohydrates, lactic acid, and fat.

Glycogen.—This substance, sometimes called animal starch, has the formula $(C_6H_{10}O_5)_n$. It is present largely in all embryonic tissues;[9]

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[1] *Ann. Chem. Pharm.* cvii. 314.

[2] *Zeit. f. anal. Chem.* vi. 33.

[3] Weidel, *Ann. Chem. Pharm.* clviii. 353.

[4] Krukenberg and Wagner, *Sitzungsber. d. physik.-med. Gesell. zu Würzburg*, 188, No. 4. Krukenberg has also examined the muscles of a large number of fishes and invertebrates for the presence or absence of the various extractives enumerated above. His results will be found in *Untersuch. des physiol. Anstalt Heidelberg*, vol. iv. Heft i.; and in *Maly's Jahresbericht*, xi. 340.

[5] *Ann. Chem. Pharm.* cxxvii. 185, cxxxiii. 300.

[6] *Ibid.* clxxviii. 266.

[7] *Zeit. rat. Med.* (3) xxxvi. 195.

[8] *Physiol. Chem.* p. 647.

[9] Claude Bernard, *Comptes rend.* xlviii. 673. Cramer (*Zeit. Biol.* xxiv. 67) obtained pure glycogen from fœtal skin and cartilage.

in the adult it is found chiefly in the liver, the muscles, and the white blood corpuscles. During life, as in the liver, the muscle glycogen appears to be converted into sugar, and this change may occur for a certain time after death. Böhm[1] considers the change into sugar does not occur till putrefaction sets in, and Demant[2] has shown that the change may be greatly hindered by the action of weak carbolic acid In estimating the amount of glycogen in muscle, the tissue should, however, be obtained as soon as possible after death, and immediately plunged into boiling water to destroy any ferment which converts glycogen into sugar. The glycogen may then be extracted with hot water (Brücke,[3] Nasse[4]), or with dilute potash (Abeles,[5] Külz[6]). If a quantitative analysis is to be made, a weighed quantity of muscle must be taken, finely divided, and repeatedly extracted until no more glycogen passes into solution. In the case of muscle especially, the dilute alkali effects a much more thorough extraction than hot water (Külz). Any proteid that passes into solution is precipitated by potassio-mercuric iodide and filtered off; the filtrate is evaporated to a small bulk and the glycogen precipitated as a white powder by excess of alcohol, or it may be converted into sugar and then estimated polarimetrically. Glycogen forms an opalescent solution in water, and gives, like dextrin, a red-brown colour with iodine. A large number of comparative estimations by weighing and by the polarimeter have been made in Külz's laboratory and found to yield very nearly equal results.[7] Cramer,[8] using Külz's method, found

1. The glycogen in the two halves of the body is equal.
2. In the heart, glycogen is unequally distributed in the different regions, so differing from the liver.
3. Different groups of muscles vary in the amount of glycogen they contain, but symmetrical or corresponding muscles contain the same amount.

Brücke[9] found glycogen in the plain muscle of the stomach; Chittenden[10] and Bizio[11] found it in the plain muscles of gastropods.

The amount of glycogen in muscle is variable; the following are the chief facts relating to variations in the amount that is present:—

1. **Influence of starvation.** The muscle glycogen in warm-blooded animals

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[1] *Pflüger's Archiv*, xxiv. 33.
[2] *Zeit. physiol. Chem.* iii. 200.
[3] Brücke, *Sitzungsber. d. k. k. Akad. d. Wissensch. Wien*, lxiii. 214.
[4] Nasse, *Pflüger's Archiv*, ii. 97.
[5] Abeles, *Med. Jahrbücher*, 1877, p. 551.
[6] Külz, *Zeit. Biol.* xxii. 161.
[7] Schmelz, *Zeit. Biol.* xxv. 180.
[8] *Ibid.* xxiv. 67.
[9] *Wiener Akad. Sitzungsber.* vol. lxiii. 2 Abth. 1871.
[10] Chittenden, *Ann. Chem. Pharm.* clxxviii. 266.
[11] Bizio, *Atti dell' Istituto Venet. di scienze*, vol. xi. (ser. 3), 1866.

disappears during inanition much more slowly than the liver glycogen (Weiss,[1] Aldehoff[2]). Luchsinger[3] stated that the heart-muscles are richer in glycogen during inanition than those of the limbs, but Aldehoff, who used Külz's method of estimating glycogen, and therefore obtained more correct results, was not able to confirm this statement of Luchsinger.

2. Influence of work. Muscular activity lessens the amount of glycogen in a muscle, it being apparently transformed into sugar (Weiss, Manché,[4] Molinari[5]). This is well illustrated by the following table (Manché).

	Weight of glycogen in limb at rest	Weight of glycogen in opposite limb, which was made to contract from 23–65 minutes	Loss of glycogen per cent. in tetanised limb
1.	0·1277 gramme	0·114 gramme	12·76
2.	0·2287 „	0·1942 „	15·09
3.	0·2267 „	0·1917 „	15·44

In other words, the limbs which were stimulated to contract lost from 12 to 15 per cent. of their glycogen in an hour. Luchsinger considered that glycogen is not a direct source of energy in contracting muscle, but this is in no way proved by his researches, for it is doubtful whether he could ever have obtained muscles free from glycogen—as we have already seen the glycogen of muscle disappears very slowly during inanition. In frogs inanition causes a rapid disappearance of the liver glycogen, but that of the muscles remains practically unaltered (Aldehoff).

3. Effect of removing the liver. Minkowski[6] and Laves[7] stated that after extirpation of the liver the muscle-glycogen markedly diminishes; they consider that the muscle-glycogen chiefly originates in the liver. C. Schmelz,[8] using Külz's method of estimating glycogen, confirms these results which were arrived at by Brücke's apparently less exact method. Schmelz, however, does not consider the point proved that the liver is the source of the muscle-glycogen, for he finds that feeding animals on cane sugar produces no marked increase of the muscle-glycogen either in normal animals or in those from which the liver has been removed. Prausnitz[9] also considers that the muscles have a glycogenic function quite apart from that of the liver.

4. Effect of cutting the nerve of a muscle. This operation causes an increase in the glycogen of the muscle (Chandelon[10]). The following table illustrates the results obtained (Manché):—

Operation of cutting one sciatic nerve, performed	Increase (per cent.) of muscle-glycogen on operated side as compared with the normal
1·5 days before death	6·25
2·7 „ „	26·67
3·20 „ „	33·33

No doubt the intact muscles of the healthy limb continue to contract after the operation, and thus lose a certain amount of glycogen; in the paralysed muscle on the other hand, the glycogen is allowed to accumulate.

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[1] Weiss, *Sitzungsb. d. k. Akad. der Wissensch.* lxiv.

[2] Aldehoff, *Zeit. Biol.* xxv. 187.

[3] Luchsinger, *Dissert.* Zürich. 1875.

[4] Manché, *Zeit. Biol.* xxv. 163.

[5] Molinari, *Chem. Centralbl.* 1889, ii. p. 572.

Minkowski, *Arch. f. exp. Path. u. Pharmakol.* xxiii. 139.

[7] Laves, *Inaug. Dissert.* Königsberg, 1886.

[8] C Schmelz, *Zeit. Biol.* xxv. 180.

[9] *Zeit. Biol.* xxvi. 377.

[10] Chandelon, *Pflüger's Archiv*, xiii. 626.

5. **Effect of cutting the tendon of a muscle.** After tenotomy the muscle appears to be in such a pathological condition that glycogen accumulates in it, and does not undergo metabolic changes so readily as in normal muscle (E. Krauss).[1]

6. Ligature of the artery supplying a muscle leads to a decrease of its glycogen, especially in those cases in which œdema follows the operation; the saturation of the tissues by lymph leading probably to saccharification (Chandelon, Manché).

Dextrin.—This is an intermediate stage in the formation of sugar from glycogen; it is difficult to distinguish it from glycogen; it has the same empirical formula, and gives the same colour with iodine; it, however, unlike glycogen, forms a clear solution with cold water. Limpricht[2] found it in horseflesh; but more extended observations by Nasse[2] have shown that its amount is variable and dependent on the stage into which the glycogen has been transformed after death.

Maltose.—It is Nasse chiefly who has worked at the transformation of glycogen into sugar. During activity and at certain stages after death the glycogen certainly diminishes in quantity, and it is believed to be changed into sugar and, according to Nasse, partly also into lactic acid. Reasons have, however, been advanced on p. 409 which show that lactic acid is probably derived from the proteids of the muscle, not from its carbohydrates. From resting muscle little or no sugar can be obtained. According to Meissner[3] the sugar which is formed on activity is not dextrose but maltose.

Inosite.—This substance, which is isomeric with dextrose but is non-fermentable, does not reduce copper salts, and has no action on the plane of polarised light, was first discovered by Scherer[4] in the heart of the ox, and has since been found in the voluntary muscles in small quantities (0·03–0·008 per cent. [Limpricht, Jacobsen]), and in unstriated muscles (Lehmann). Inosite is also found in other animal tissues and in many plants. It crystallises in colourless monoclinic tables ($C_6H_{12}O_6 + 2H_2O$), and when pure gives with nitric acid and calcium chloride a pink colour (*see* p. 100).

Alcohol.—Small quantities of ethyl alcohol were found by Rajewsky[5] in the fresh muscles of the rabbit, ox, and horse. Béchamp[6] confirmed this observation.

Lactic acid.—The question of the method of the formation of lactic acid has already been discussed in connection with *rigor mortis*

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[1] E. Krauss, *Virchow's Archiv*, cxiii. 315.

[2] *Loc. cit.*

[3] Meissner, *Göttinger Nachrichten*, 1861, p. 206, and 1862, p. 157.

[4] Scherer, *Ann. Chem. Pharm.* lxxvii. 322. For the most recent account of the chemical constitution of inosite *see* Maquenne, *Compt. rend.* vol. civ. (1887), pp. 225, 297, 1719, 1858.

[5] *Pflüger's Archiv*, xi. 122.

[6] *Compt. rend.* xxxix. No. 18.

(*see* p. 409), and the opinion has been advanced that it is formed from proteid, not from carbohydrate. This acid is produced during both the contraction and the death-rigor of muscle.

We have already seen that there are two isomeric lactic acids ($C_3H_6O_3$) produced; one is sarcolactic or para-lactic acid (optically active ethidene lactic acid), and the other is ethene lactic acid. We have still to describe the preparation and distinctive properties of these acids.

Preparation.—The syrupy liquid from which creatine has crystallised (Liebig's method, *see* p. 419) is acidulated with sulphuric acid and extracted with ether. The ether contains the lactic acids in solution; it is evaporated to dryness and the residue dissolved and boiled in water in which carbonate of zinc is suspended. It is then filtered, and the filtrate evaporated to a small bulk. On treating this with absolute alcohol, the liquid deposits needles of zinc sarcolactate, the ethene lactate of zinc remaining in solution. The sarcolactate is filtered off, and the filtrate evaporated down, when the ethene lactate separates out. This also is filtered off. We thus have zinc sarcolactate on one filter, and zinc ethene lactate on another. The free acid is prepared from each in the same way. The crystals of the zinc salt are dissolved in water; sulphuretted hydrogen is passed through the solution; the zinc sulphide is filtered off; the filtrate is concentrated, shaken with ether, and, on evaporating the ethereal extract to dryness, the free acid is obtained.

Properties of the isomeric lactic acids.

Sarcolactic acid	Fermentation lactic acid from milk	Ethene lactic acid
1. Dextrorotatory ($\alpha = +3{\cdot}5$).	1. Optically inactive	1. Optically inactive.
2. Zinc compound has the formula $Zn(C_3H_5O_3)_2 + 2H_2O$. It loses all its water of crystallisation (12·9 per cent.) at 100° C. Specific rotation $= -7{\cdot}6°$. Very insoluble in alcohol. Soluble in 17·5 parts of water at 14° C.	2. Zinc compound has the formula $Zn(C_3H_5O_3)_2 + 3H_2O$. It loses all its water of crystallisation (18·18) at 100° C. Optically inactive. Insoluble in alcohol. Soluble in 58–60 parts of water at 14° C.	2. Zinc compound has the same formula as the sarcolactate. Optically inactive. More soluble in alcohol. Very soluble in water.
3. Calcium compound has the formula $2[Ca(C_3H_5O_3)_2] + 9H_2O$. Specific rotation $= -3{\cdot}8°$.	3. Calcium compound has the formula $Ca(C_3H_5O_3)_2 + 5H_2O$. Optically inactive.	
4. When oxidised with dilute chromic acid, acetic and formic acids are produced. At 100° lactic anhydride ($C_6H_{10}O_5$) and at 140° lactide ($C_3H_4O_2$) are formed.		4. Oxidised with dilute chromic acid, malonic acid ($C_3H_4O_4$) is produced. Lactic anhydride and lactide are formed as in the case of the two other acids.

Quantity of lactic acid in muscle.—This is variable; the various analyses that have been made give numbers varying from 0·1 to 1·0 per cent. (Jacobsen, Takacs, Böhm, Demant).

Fat.—A certain quantity of fat is always present between the muscular fibres; it is not possible to say whether any of the fat obtained from muscle comes from the muscular substance proper or not.

There are two conditions, however, in which fat is undoubtedly present:—

1. In the affection known as fatty degeneration, the interior of the sarcolemna becomes crowded with fat granules and globules; these first obscure and finally obliterate the striations of the contractile substance. It often occurs markedly in the heart; it may be produced artificially by certain poisons, especially by phosphorus.

2. After death the muscular substance may be replaced by a waxy material, known as *adipocere*. This occurs especially in corpses buried in damp soil, or in bodies which remain in water some time after death. The length of time after death that these changes occur has been the subject of extended observations, especially at the Paris Morgue; it is found to occur in the muscles in a definite order, and the amount of adipocere present is a very good gauge of the time a body has been dead.[1] Adipocere consists chiefly of the calcium soaps of palmitic and stearic acids, and, in some cases, of acid ammonium soaps also.[2] Hoppe-Seyler[3] regards the change as a result of a ferment action.

The formation of fat from proteids probably occurs under other circumstances; for instance, fat is formed in the body on an exclusively proteid diet.

Inorganic Constituents of Muscle

The most noteworthy points in the inorganic constituents of muscle are the predominance of potassium over sodium among the bases, and of phosphoric acid among the acids. This appears to be a general rule throughout the animal kingdom. The total amount of ash is from 1 to 1·5 per cent.

The following analyses are by Bunge[4]:—

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[1] *See* more fully in works on Forensic Medicine.

[2] Quain, *Med. Chir. Trans.* 1850, 141. Virchow, *Verhandl. d. phys. med. Gesellsch. zu Würzburg*, vol. iii. Wetherill, *Journ. f. prakt. Chem.* vol. lxviii. p. 26. K. B. Lehmann, *Bied. Centralbl.* 1889, p. 66.

[3] *Physiol. Chem.* p. 119.

[4] Bunge, *Zeit. physiol. Chem.* ix. 60. Other analyses will be found in Hoppe

In parts per 1,000

	I.	II.
K_2O	4·654	4·160
Na_2O	0·770	0·811
CaO	0·086	0·072
MgO	0·412	0·381
Fe_2O_3	0·057	—
P_2O_5	4·644	4·58
Cl	0·672	0·70
SO_3	—	0·10

The Gases of Muscle

The subject of the gaseous constituents of muscle is one at which a large amount of painstaking work has been done. The forms of apparatus used have been already described (p. 31), and we shall now consider the principal results which have been arrived at. In discussing this subject it will be necessary to draw distinctions between active and resting muscle, and also to consider the gases of the blood which is entering and of that which is leaving the muscle.

Gases of the muscle itself.—In order to extract the gases from muscle (the muscle *in vacuo*) care must be taken to avoid entanglement with air ; it is necessary to use a form of mercurial air-pump much like those employed in extracting the gases from the blood ; the boiling-flask is perforated with platinum wires so that, if necessary, the muscle may be excited to contract while *in vacuo*, and the froth-chamber is so arranged that an acid can be made to pass when desired from it to the muscle (fig. 15, p. 32).

Muscle which has been freed from blood and removed from the body, then scalded to prevent rigor, and minced, yields a small quantity of carbonic acid ; this is increased on adding acid, by the liberation of the gas from carbonates. Hermann's [1] results were :—

Free carbonic acid	2·74 per cent.
Fixed carbonic acid	1·95 „

Muscles in which *rigor mortis* has been prevented by freezing and placed in the boiling-flask containing boiled salt solution give off no gas at 0° C., but above this temperature, especially when rigidity sets

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Seyler's *Physiol. Chem.* pp. 650, 651. They, however, simply illustrate the same points.

[1] Hermann (*Untersuchungen ü. d. Stoffwechsel der Muskeln ausgehend vom Gaswechsel.* Hirschwald, Berlin, 1867).

in, there is a discharge of gas, and, if putrefaction be allowed to supervene, a further discharge. In the portions of gas first set free small quantities of nitrogen are constantly found, but oxygen is always absent. The following numbers were obtained by Hermann in one experiment:—

Free carbonic acid liberated at 60° C. . .	11·79 per cent.
Fixed carbonic acid, i.e. liberated by acid .	2·04 „
Nitrogen	1·23 „
Oxygen	0·0 „

There is thus a great increase in the amount of carbonic acid as compared with scalded muscle, i.e. muscle in which rigor does not occur.

The following experiment illustrates the effect of contraction; again there is an increase in the discharge of free carbonic acid; no oxygen is ever found; a small admixture of nitrogen may be neglected.

	Resting muscle	Tetanised muscle
Free carbonic acid . .	3·01 per cent.	7·66 per cent.
Fixed carbonic acid .	4·90 „	4·42 „

Stuitzing[1] showed that on the prolonged boiling of muscle a substance is broken up which yields a large amount of carbonic acid; this substance is probably the same as that which is decomposed with the production of carbonic acid on tetanus or rigor, as, after tetanus or rigor, boiling does not produce nearly such a large yield of the gas.

Muscular respiration. The muscle in the air. The simplest method for investigating the changes produced in air by the presence of resting or contracting muscle is that of Hermann. The muscle is placed in a certain quantity of air in a graduated tube over mercury.

Electrodes can be placed in contact with the muscle and so contraction produced, and at the end of the experiment the muscle can be withdrawn through the mercury. The gases left in the tube are first dried, the carbonic acid absorbed by potash, the oxygen exploded with hydrogen or absorbed by pyrogallic acid, and the oxygen of the original air calculated from the volume of nitrogen left behind.

The chief results obtained are as follows: –

1. A small amount of oxygen is absorbed. This occurs both in resting, active, and rigid muscle, and is chiefly due to the onset of putrefaction. A small quantity of the oxygen is, however, probably absorbed by the muscle for its vital processes. We have seen that no oxygen is obtainable from a muscle; the oxygen taken from the air, or,

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[1] *Pflüger's Archiv*, xxiii. 388.

when the muscle is in the body, from the blood, is not present in the free condition, but in a state of combination. This compound is a firmer one than oxyhæmoglobin, and holds the oxygen until it is required for the oxidative changes that occur when a muscle contracts. A muscle will, as we have seen, contract *in vacuo*; a heart will continue to beat for some time in a chamber containing no oxygen. This is in virtue of the oxygen stored up in the muscular tissue itself. When carbonic acid is formed in a muscle it is not due to direct oxidation; and hence, when one finds that a small quantity of oxygen is taken up by a muscle in such experiments as Hermann's, it must be clearly understood that what is meant is that this oxygen is entering into combination with something that holds it in the muscle for future use, not for the immediate formation of carbonic acid, water, and other products of oxidation.

2. While the quantity of oxygen absorbed remains practically constant, the amount of carbonic acid given off is much increased by contraction or on the onset of rigidity. This may be best illustrated by experiments.

		Oxygen absorbed	Carbonic acid given off
1.	Living muscle	18 per cent.	8 per cent.
	Rigid muscle	16 „	16 „
2.	Muscle at rest	6 „	1 „
	Tetanised muscle	8 „	9 „

Muscular respiration. The muscle in the body.—The gases in the blood entering and leaving the muscle are analysed first while the muscle is at rest; and then when the muscles are made to contract.

Taking arterial blood as the standard, Ludwig and Schmidt[1] obtained the following numbers:—

Venous blood	Oxygen less than arterial blood	Carbonic acid more than arterial blood
Muscles at rest	9 per cent.	6·7 per cent.
Muscles in action	12·26 „	10·8 „

The difference is really more considerable than the table shows, because, when muscles contract, the blood flow is accelerated in the veins.

Another point is noticed in analyses of this kind, and it is this, that the ratio between the increase of carbonic acid and the decrease of oxygen is greater during contraction than during repose.

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[1] *Arbeiten aus der physiol. Anstalt in Leipzig*, 1868, vol. iii. p. 1.

In the above experiment, let Q be the relation when the muscles are at rest; and Q′ when they are in action.

Then $Q=\frac{6\cdot7}{9}$ and $Q'=\frac{10\cdot8}{12\cdot26}$; and $Q'>Q$.

In other words, more carbonic acid is produced than oxygen is absorbed: this is again what we have previously seen in contracting muscles in their effect upon the gases of the atmosphere. The increase in the quantity of oxygen absorbed in such experiments is partly due to the acceleration of the blood stream during contraction. If, however, the blood be first artificially deprived of its oxygen, before being sent to a muscle, the muscle loses its irritability, at first slowly, afterwards more quickly. A very minute quantity of oxygen will however restore irritability.

Of other changes in the blood produced by contraction of muscle, little or nothing is known; during tetanus, the venous blood is said to acquire sarcolactic acid (Spiro)[1]; and a certain quantity of reducing substances of unknown nature (Schmidt; *see* p. 433).

CONTRACTION OF MUSCLE (SUMMARY)

When muscle contracts, it undergoes both chemical and physical changes. The latter, the physical changes, consist in a conversion of the potential energy of chemical affinity into various forms of actual energy: (1) mechanical motion due to the shortening and widening of the muscular fibres; (2) heat: the rise in temperature can be seen and measured by means of thermoelectric apparatus; (3) changes of electrical potential: contracted muscle is negative to uncontracted; in this point contracted muscle resembles injured and dead muscle. Accompanying these changes, there are alterations in the optical appearances of the muscular fibres, which have already been briefly described (p. 402); and there are also changes in the extensibility and elasticity of the muscle, the most striking of these being an increase of the extensibility of contracted as compared with uncontracted muscle; that is to say, a given weight will stretch a contracted muscle more in proportion to its length than it will the same muscle in a state of rest.

The chemical changes that occur when a muscle contracts are similar in kind to those that occur when the muscle is at rest; on contraction there is a sudden acceleration and extension of these chemical decompositions. A healthy muscle connected to the central nervous system becomes longer when the nerve connecting it is cut, or when the end-plates by which the nerve fibres communicate with the muscular fibres are poisoned by curare. A healthy muscle at rest is thus contracted or retracted to a certain small degree; the small amount of contraction in such a resting muscle is called *tonus*; and when this is diminished by severance of the muscle from the central nervous system, the chemical changes occurring during rest (chemical tonus) are lessened also. This is indicated by the accumulation of glycogen in such muscles, and by the fact that their venous blood contains less carbonic acid than the venous blood of normal resting muscles does.[1] We can now proceed to give a *résumé* of the chemical differences between contracted and resting muscle.

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[1] *Zeit. physiol. Chem.* i. 111.

1. *Changes in the proteids.*—There has been no evidence adduced as yet to show that any changes in the proteids occur when a muscle contracts. Hermann's theory of muscular contraction assumes that a change occurs similar in kind, though less in degree, to that which occurs on the death of the muscle. But anything like the formation of a clot of myosin during the contraction of a muscle has never been actually observed. We shall presently see that muscular activity has very little influence on the amount of nitrogen excreted in the urine, and this supports the idea that the proteids undergo little or no increased combustion during muscular contraction.

2. *Change in reaction.*—There is, however, during muscular contraction an increased formation of the lactic acids, so much so, that the muscle acquires a distinctly acid reaction to litmus paper. Resting muscle is either alkaline or neutral. Tetanised muscle is acid, and the more vigorously muscle contracts on stimulation, the more rapidly does it become acid; if for instance a slowly contracting muscle from a tortoise and a rapidly contracting muscle from a frog be tetanised for an equal time, the acidity in the latter case is greater than in the former. The same contrast holds for the slowly contracting red muscles, and the rapidly contracting pale muscles of the rabbit (Gleiss[2]).

In the formation of lactic acid, the contraction of living muscle resembles what occurs upon the death of muscle (*rigor mortis*).

3. *Changes in the extractives.*—During tetanus sugar is produced presumably from glycogen which diminishes in quantity (Ranke,[3] Nasse[4]). During tetanus the extractives soluble in water decrease, and those soluble in alcohol increase (Helmholtz,[5] Ranke,[3] Heidenhain

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[1] Claude Bernard, *Leçons sur les propriétés des tissus vivants*, p. 221, Paris, 1866. Pflüger (*Pflüger's Archiv*, xviii. 302) arrived at the same result as Claude Bernard, not by actually examining the blood, but by estimating and comparing the amount of carbonic acid in the expired air from curarised and non-curarised animals.

[2] W. Gleiss, *Pflüger's Archiv*, xli. 69.

[3] Ranke, *Tetanus*, Leipzig, 1865.

[4] Nasse, *Pflüger's Archiv*, ii. 97.

[5] Helmholtz, *Arch. f. Anat. und Physiol.* 1845, p. 72.

with Nigetiet and Hefner[1]). This seems to be dependent in part at least on the diminution in the quantity of glycogen, and increase in the amount of sugar. With regard to the relative amount of creatine and creatinine in resting and contracting muscle, various conflicting statements have been made (*see* p. 421); but the balance of evidence is very much in favour of the view that on contraction creatine is transformed into creatinine.

4. *Production of reducing substances.*—Resting muscle oxidises pyrogallic acid, tetanised muscle does not. A solution of nitrites passed through contracting muscle is changed into nitrates, and the colour of solutions of indigo-sulphate is altered in the same way as it is by reducing substances (Grützner,[2] Gschleidlen[3]). A. Schmidt[4] arrived at similar conclusions from the examination of the venous blood of tetanised muscle, but what the reducing substances are that are produced is quite unknown.

5. *Changes in the salts.*—Weyl and Seitler[5] have pointed out that in the earlier stages of muscular activity the acid reaction may be partially due to acid potassium phosphate produced from the alkaline phosphate, by the development of new phosphoric acid from organic phosphorised compounds like lecithin and nuclein in the muscle.

6. *Changes in the gases.*—These may be briefly summarised by saying that on contraction, as on the occurrence of *rigor mortis*, the amount of carbonic acid given off is increased. The amount of oxygen absorbed is also increased, but not in proportion; in other words, the fraction $\frac{\text{Carbonic acid exhaled}}{\text{Oxygen absorbed}}$ is increased.

Fatigue of muscle.—The way in which repeated contraction causes what is known as fatigue is very uncertain. It may be due to the accumulation of the products of combustion, or to a defect of oxygen, and probably of other constituents of a normal muscle; or it may be due to a combination of these two sets of causes.

With regard to the former, the accumulation of products of combustion, Ranke[6] pointed out the depressing effect on muscular irritability produced by all acids, carbonic acid, and lactic acid, among others. Renewal of the blood stream through exhausted muscles causes them to revive; this may be due (1) to the removal of the acids and other products of contraction; (2) to the fresh supply of oxygen; or (3) more probably still to both these factors combined. Mosso[1] considers that the poison which causes the symptoms of exhaustion is probably not carbonic acid, but a substance produced in small quantities of an alkaloidal nature.

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[1] *Pflüger's Archiv*, iii. 574.

[2] Grützner, *Ibid.* vii. 255.

[3] Gschleidlen, *Ibid.* viii. 506.

[4] *Sitzungsberichte der k. k. Akad. Wien*, vol. xx.

[5] *Zeit. physiol. Chem.* vi. 557.

[6] J. Ranke, *Tetanus*, p. 350. The increased acidity of fatigued muscles has since been noted by numerous observers; among the more recent researches on the subject may be mentioned: Warren (*Pflüger's Archiv*, xxiv. 391), who finds that, though the acidity is increased, the number of acid molecules in the muscle are diminished; this is explained by supposing that in resting muscle the anhydride, and in contracting muscle the free acid is present, which latter combines with twice the amount of base as the anhydride; Moleschott and Battistini (*Arch. Italiennes*, viii. 90) used phenol phthalein and

Hermann's theory of muscular contraction.[2]—The fact that no oxygen is obtainable from muscle suggested to Hermann the theory that the formation of carbonic anhydride on contraction is not simply due to oxidation, but rather to the decomposition of a complex substance with the formation of certain simpler products of which carbonic anhydride is one. This complex nitrogenous substance he calls *inogen*, and the products of its decomposition he considers to be carbonic anhydride, sarcolactic acid, and myosin. The first passes into the blood stream, and the other two apparently help in forming again the original inogen. Hermann considers that the same decomposition occurs in *rigor mortis*, only to a much greater extent; contraction is thus a condition of partial death, and this view is supported not only from the chemical standpoint (the formation of acid), but also from the electrical point of view, both dead and contracted muscle being negative

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potash as indicator; A. Monari (*Chem. Centralbl.* 1887, p. 1360) considers that the increased acidity causes a partial conversion of creatine into creatinine. Marcuse (*Pflüger's Archiv*, xxxix. 425) considers that whereas the lactic acid formed during *rigor mortis* is not formed from glycogen (p. 409), yet that formed during contraction probably is, as the glycogen diminishes on contraction. But, as Molinari points out (*Chem. Centralbl.* 1888, vol. ii. p. 372), this is by no means conclusive, as the diminution of glycogen on contraction is probably due to its conversion into sugar. Marcuse also finds that the urine of frogs after extirpation of the liver contains lactic acid if the muscles be tetanised. Under normal circumstances the liver destroys lactic acid (Minkowski).

[1] *Report of Internat. Med. Congress*, Berlin, 1890.

[2] For an account of the various theories that were held regarding muscular contraction previous to Hermann, the reader is referred to Gamgee's *Physiol. Chem.* pp. 406-413. John Mayow (1668–1674), who really discovered oxygen, spoke of it as nitro-aërial particles, and the combination of these with salino-sulphureous or combustible particles in the muscle caused heat, and an effervescence which produced muscular contraction. Stahl introduced the doctrine of an immaterial anima with unlimited spontaneous power over matter, including the muscles; this under the names of sentient principle (Whytt) and vital force (Hunter) survived until Liebig's time. The rediscovery of oxygen and of its importance to animal life was the occasion of the production of several theories as to the part it played in causing contraction of muscles; and the theory of the conservation of energy helped in the understanding of the relations of the physical and chemical changes which occur in muscle (Mayer, Heidenhain). M. Traube pointed out fully that no albuminous substance is used up on contraction, and Matteucci concluded that the oxygen which forms the carbonic acid in muscular respiration is derived, not from the air or blood directly, but from that which is present in the muscle itself in a state of loose chemical combination.

to living muscle at rest. The question arises, is there any evidence of the formation of a muscle-clot (myosin) during contraction? It is difficult to prove or disprove such a contention, as Hermann supposes that the process of clotting is not so complete as in *rigor mortis*, but that it only goes as far as the viscous or gelatinous stage, and such a change would not be apparent to the microscope.

I have in the earlier parts of this chapter pointed out that myosinogen and myosin are easily converted the one into the other (p. 408), and a suggestion that might arise is this—if myosin can be made to clot and unclot so easily out of the body, is it not possible that a similar condition exists in the body? The experiments certainly show that myosin and myosinogen are very unstable substances; and this is supported by the fact that myosinogen acts like fibrin-ferment in hastening blood-coagulation; it is therefore quite possible that during contraction the proteid myosinogen may undergo certain intra-molecular rearrangements, perhaps of the same nature as those which occur to a far greater degree on the death of the muscle, in each case leading to a liberation of acid. But with regard to the formation of a clot during contraction, there is one physical change which in particular shows there is a great distinction between dead muscle and contracted living muscle. This change is the alteration in the extensibility of the muscle, which in *rigor mortis* is diminished and on contraction is increased. In other words, *rigor* makes the muscle less extensible, because it becomes more solid owing to the formation of the myosin clot, but, during the contraction of a living muscle, it becomes in a sense more liquid, as is shown by its increased extensibility, and this is certainly against the theory of the formation of a solid clot during contraction.

Bernstein's theory of muscular contraction.[1]—The following admirable summary of Bernstein's views is taken from Dr. Burdon-Sanderson's address to the British Association, 1889.[2] 'The contraction which a muscle undergoes when stimulated has its seat, not in the system of inotagmata (*see* p. 189, or disdiaclasts, *see* p. 403), but in the interstitial material that surrounds it, and consists in the migration of that labile material from pole to equator, this being synchronous with explosive chemical change, sudden disengagement of heat, and change in the electrical state of the living substance. The chemical changes that take place and lead to the production of heat are indices of oxidation. There must be in the sphere of each tagma an accumulation of oxygen and oxidisable material, and concomitantly with or antecedently to the migration of liquid from pole to equator these must come into encounter. Let us suppose that a soluble carbohydrate is the oxidisable material, and that this is accumulated equatorially, and oxygen at the poles; consequently, between equator and poles, water and carbonic acid, the products of the explosion are set free. That the process is really of this nature is the conclusion to which an elaborate study of the electrical phenomena which accompany it has led Professor Bernstein. His view of the molecular structure of muscular protoplasm is in entire accordance with the theory of Pflüger (*see* p. 115), and with Engelmann's scheme of muscular structure (*see* p. 189), with this addition, that each inotagma is electrically polarised when in a state of rest, depolarised at the moment of excitation or stimulation, and that the axes of the tagmata are so directed that they are always parallel to the surface of the fibre and consequently have their positive sides exposed. In this amended form the theory admits of being harmonised with the fundamental facts of muscle-electricity, namely, that cut surfaces are negative to sound surfaces, and excited parts to inactive, provided that the direction of the hypothetical polarisation is from equator to pole, i.e. that in the resting state the poles of each tagma are charged with negative ions, the equator with positive, and consequently that the direction of the discharge in the catalyte (or oxidisable material) at the moment that the polarisation disappears is from pole to equator. The same theory, moreover, enables us to express more consistently the accepted explanations of many collateral phenomena, particularly those of electrotonus. Sufficient has, however, been said to show that it is not impossible to regard the three phenomena (chemical explosion, sudden electrical change, and change of form) as all manifestations of one and the same process—as products of the same mechanism.'

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[1] Bernstein, 'Neue Theorie d. Erregungsvorgänge u. electr. Erschein. an der Nerven- und Muskelfasern,' *Unters. aus dem. Physiol. Institut*, Halle, 1888.

[2] *Reports Brit. Assoc.* 1889.

Effects of Muscular Contraction on the Urine

The effects of muscular contraction on the urine are exceedingly slight; it is only after prolonged and violent muscular exercise that any change can be perceived at all, and even then it is out of all proportion to the amount of contraction. This is in marked contrast to the very great effect that muscular contraction has on the respiratory excretion (*see* pp. 374, 427). It is in the urine that urea, uric acid, and other products of nitrogenous metabolism or combustion leave the body; and, as we have already seen, there is very little change in the chief nitrogenous constituents of muscle, the proteids, on contraction; but the substances which undergo an accelerated chemical change or combustion on contraction are the non-nitrogenous constituents.

Voit[1] investigated the question in a dog; the animal either went without food or was put on a carefully regulated, fixed diet; the work done was the turning of a treadwheel, and the urine was carefully collected. A very slight increase in the amount of urea excreted was noted after work, and it was not at all proportional to the amount of work done.

The experiments made on human beings fully corroborate Voit's experiments. Of these the experiment of Fick and Wislicenus[2] in the ascent of the Faulhorn has become classical. The following are the chief of the facts they ascertained.

For seventeen hours before the ascent, during the ascent, and for some hours afterwards no nitrogenous food was taken. The urine was carefully collected and the urea determined in it by Neubauer's method for the periods before, during, and after the exertion. The work done was also estimated. The total nitrogen was determined by combustion with soda-lime. The height of the mountain is 1,956 metres. Fick weighed 66 kilograms, and Wislicenus 76 kilograms. The work done by Fick in raising his body to the top of the mountain $=66 \times 1956 = 129{,}096$ kilogram-metres; similarly, the work done by Wislicenus was 148,656 kilogram-metres. This does not take into account any other muscular work, such as the movements of respiration, circulation, movements of the arms and trunk muscles, &c. The nitrogen in the urine (the small quantity which escaped by the sweat and fæces being disregarded) during the work and for six hours after was, in the case of Fick, 5·7 grammes, which corresponds to that obtained from 37·1 grammes of proteid; and, in the case of Wislicenus, to 5·5 grammes, which corresponds to that obtained from the decomposition of 37 grammes of proteid.

Frankland[3] has shown that from the burning of 1 gramme of lean beef a quantity of heat is formed which corresponds to 2161 kilogram-metres, so that the amount of work obtainable from 37·1 grammes of proteid in Fick's case was $37{\cdot}1 \times 2161 = 80{,}324$ kilogram-metres; in Wislicenus' case $37 \times 2161 = 79{,}956$ kilogram-metres—that is to say, much less than the work actually done. The disproportion is really greater, because the physiological heat-value of proteid is less than its physical heat-value; proteids do not in the body undergo complete combustion; the physiological heat-value of proteid is its physical heat-value *minus* the heat-value of urea.

This experiment clearly showed that proteid metabolism will not account for all the work done; it however does not settle the question as to whether the nitrogen excreted is increased by work; it so happened in the actual experiment that the nitrogen excreted was lessened as compared with the periods before and after the muscular exertion, but the conditions under which the experimenters worked were not sufficiently rigorous to admit of accurate comparisons being drawn.

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[1] *Untersuch. ü. d. Einfluss des Kochsalzes, des Kaffees und der Muskelbewegungen auf den Stoffwechsel*, Munich, 1860.

[2] *Vierteljahresschrift d. naturf. Gesellsch. in Zürich*, x. 1865. *London, Edin. und Dublin Phil. Magazine*, series 4, vol. xxxi. p. 485.

[3] Frankland, *Lond. Edin. and Dublin Philos. Mag.* series 4, vol. xxxii. p. 187.

The question as to the influence that work has on the increase or decrease of the nitrogen excreted has been investigated by Parkes[1] on soldiers; he found a slight increase during work; by Flint[2] and Pavy[3] on the pedestrian Weston, who arrived at contradictory results; and by North,[4] who experimented on himself. The last-named experiments are by far the most thorough that have been made; the following is a *résumé* of the methods employed and the results obtained.

Each experiment lasted nine days; four days of ordinary occupation, one day's work, and a second period of four days of ordinary occupation. 'Reserve nitrogen' was got rid of by thirty-six hours' abstention from food, or by severe labour before the commencement of the experiment. Observations were made twice daily on the pulse, rate of respiration, temperature of body, and body-weight. The food, carefully analysed, weighed, and cooked by the experimenter himself, was taken in four meals, and consisted of bread specially made by Mr. North himself, dried meat-powder, 'desiccated potato,' 'dried julienne,' condensed milk, cocoa, 'American evaporated apples,' 'Australian beef marrow,' sugar, salt, tartaric acid, and sodium carbonate (for raising the bread).

None of these articles of food presented any difficulties as regards analysis. The food, of which there was an unlimited supply, was of *constant composition*, so that for the first time in such experimentation a food, the chemical composition of which was absolutely known, was used. The fæces and urine were collected in specially-prepared bottles, carried in a knapsack during walking, which was the special form of work selected. The nitrogen (estimated by combustion with soda-lime), chlorides, sulphates, and phosphates were estimated both in food and excreta. For full particulars, the original memoir must be consulted, but the following summary of the full tables from one of the experiments will serve as an example to illustrate the general results obtained.

The experiment lasted from June 7–15, 1882; June 11 was the day on which work was done. The work was a walk of 32 miles in seven hours, the load carried being 27·75 lbs., and the loss of body-weight after the walk, 4·5 lbs.

Averages per diem

Date	Urine			Fæces	
	P_2O_5	H_2SO_4	N	P_2O_5	N
June 7–10	1·97	2·75	13·78	2·17	2·26
„ 11	1·98	3·65	16·15	—	—
„ 12	1·86	3·02	16·31	2·77	2·57
„ 13–15	1·71	2·74	14·68	2·25	2·87

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[1] Parkes, *Proc. Roy. Soc.* xi. 339; xvi. 44.

[2] Flint, *Journal of Anat. and Physiol.* xii. 91.

[3] Pavy, *Lancet*, 1876 (numerous papers).

[4] North, *Journal of Physiol.* i. 171. *Proc. Roy. Soc.* xxxix. 443.

Daily balance of Ingesta and Excreta.

Date	N of ingesta	N of excreta	Difference	Total P_2O_5 in excreta daily
June 7	17·64	16·28	1·36	3·54
„ 8	35·28	32·76	2·52	4·68
„ 9	52·92	48·41	4·51	4·37
„ 10	70·57	60·90	9·67	1·80
„ 11	88·21	79·62	8·59	4·75
„ 12	105·85	98·40	7·45	3·94
„ 13	123·50	116·19	7·31	4·25
„ 14	141·14	133·68	7·46	3·51
„ 15	158·78	150·65	8·13	4·50
	Total P_2O_5 excreted			35·34
	„ „ ingested			34·84
			Difference . .	0·50

Per diem	Before work	After work	Difference
Nitrogen in excreta	15·22	17·95	2·73
P_2O_5 in excreta	3·59	4·19	0·60
H_2SO_4 in urine	2·74	2·97	0·23

The weights in the foregoing tables are expressed in grammes. The results seen are as follows: *nitrogen*—obvious increase on the day of work continued on the days following it. The reserve at the end of the experiment was only 1·54 gramme less than on the day before the work: *phosphoric acid*—the excess of P_2O_5 excreted over that ingested (0·5 gramme) is probably within the limits of experimental error: *sulphuric acid*—the increase after the work is undoubted, and proportional to the increase of nitrogenous material excreted: the amount of sulphates in the food was insignificant, and that in the urine was therefore derived from proteid metabolism.

These results confirm those of Parkes, but the disturbance produced by very severe labour was much more immediate and of greater intensity than that which Parkes observed, probably because the exertion he imposed on the soldiers under observation was inadequate. As in Parkes's experiments, where retention of nitrogen followed the diminution of nitrogen stored in the body, produced by privation of nitrogenous food, so after the disturbance of nutrition produced by severe labour, the immediate effect of which is to diminish the store of nitrogenous material in the system, there follows a corresponding diminution of discharge, the output being less than the intake. This store of nitrogen is more constantly operative than has been hitherto supposed; thus accumulation took place when the daily supply of nitrogen was not more than 17·6 grammes, no extra work being imposed; this amount cannot be regarded as more than an adequate supply for the normal needs of the body. The retention following starvation or exercise is a mere exaggeration of the normal tendency.

Muscular contraction thus enlarges the total excretion of nitrogen, but the increase is very small and is out of all proportion to the work done or the body-weight lost during the exercise. No doubt the nitrogen eliminated is derived ultimately from the muscles; but, as

North's experiments show, it is rather from what may be called reserve-nitrogen that the increased output is derived, not from the muscular nitrogen direct. As Gamgee[1] points out, the effete nitrogen may leave the muscle not as urea or any intermediate substance in the formation of urea, such as creatine, but as proteid, and this proteid may be oxidised to form urea somewhere else.

APPENDIX

ELECTRICAL ORGANS

About fifty species of fishes are believed to possess electrical organs; the best known of these are the torpedo ray, the common skate, the electric eel (Gymnotus), and the Malapterurus. Many interesting physiological observations have been made upon these organs by Du Bois Reymond and by Burdon-Sanderson, Gotch, and Ewart with regard to their histology, development, and electromotive phenomena.[2] In Malapterurus the organ appears to be epithelial in origin, but in other cases the organ appears to be analogous to muscle or to be developed from embryonic structures which elsewhere lengthen into muscular fibres; the nerve terminations, which in muscle form the comparatively small end-plates, in the electric organ form more extensive expansions.

But very little chemico-physiological work has been done at this subject; the observers speak of a mucoid fluid in the spaces between the electrical plates; and from the torpedo organ Weyl[3] has extracted a substance which gives the reactions of mucin, except that no reducing sugar can be obtained from it on treatment with dilute acids; he calls it torpedo-mucin. A small quantity of gelatin and of a globulin (coagulated by heat at 55°–60°) was also obtained. The heat-coagulation temperature of the globulin is the same as that of myosinogen;[4] and it is also interesting to note, when comparing the organ with muscle, that, like muscle, it becomes acid after death (Boll),[5] and much less transparent.

Weyl[6] found the percentage of water in the muscles of torpedo to be 77·5, in the electrical organ 89. He was also able to separate a number of organic substances from the electrical organ similar to those occurring in muscle and nerve, such as creatine, xanthine, lecithin, fat, cholesterin, fatty acids, and inosite. Frerichs and Städeler found urea.

In another research[7] Weyl found that excitation of the organ produced an increased formation of phosphoric acid in it.

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[1] *Physiol. Chem.* p. 409.

[2] McKendrick's *Physiology*, vol. i. chap. xx. 1888, contains a *résumé* of these researches, with bibliography. Du Bois Reymond's papers are translated in the *Oxford Biological Memoirs*, vol. i. 1887. *See* especially on the Chemical Reaction of the Electrical Organ of Malapterurus, p. 412. (It becomes acid on activity.)

[3] Weyl, *Zeit. physiol. Chem.* xi. 525.

[4] Krukenberg ('Weitere Untersuch. zur vergleich. Muskelchemie,' *Vergleich. physiol. Studien*, 2 Reihe, 1 Abth. pp. 143–147) states, however, that he was unable to obtain myosin from the electrical organ of torpedo.

[5] *Arch. für Anat. und Physiol.* 1873, p. 99.

[6] *Monatsber. d. königl. Akad. d. Wissensch. zu Berlin*, April 1881.

[7] Du Bois Reymond's *Archiv, physiol. Abth.* 1884, pp. 316–324.

CHAPTER XXI

EPITHELIUM

Epithelium may be defined as a tissue which consists entirely of cells united by a small amount of cementing substance. As a rule an epithelium is spread out to form a membrane, lining a cavity or covering a surface. But in certain cases the tissue is not spread out in this way; for instance, the liver is an organ which may be said to consist of a mass of epithelial cells, and the various forms of cancer are also epithelial growths.

Epithelia may be classified from the histological standpoint into those which consist of one layer of cells only, called simple epithelia, and those which consist of more than one layer, which are termed compound.

The simple epithelia may be again subdivided into pavement (or endothelium), columnar, cubical, and ciliated, according to the shape of the component cells.

The compound epithelia comprise the transitional epithelium of the bladder and ureters, and the stratified epithelium, such as that lining the mouth, or covering the whole of the external surface of the body, where it is called the epidermis.

Separated from these various forms of epithelium on account of their specialised functions, the two following must be mentioned: secreting epithelium, such as occurs in the alveoli of the salivary glands, or the uriniferous tubules; and nerve-epithelium, the various forms of modified epithelial cells which are connected to the terminations of various sensory nerves, and form the receptive end organs for sensations of different kinds; instances of nerve-epithelium are the rods and cones of the retina, the auditory hair-cells, the olfactorial cells, &c.

Very little or nothing is known chemically with regard to a great number of the varieties of epithelium just enumerated. Microscopic research shows that the constituent cells are protoplasmic and contain nuclei, and we conclude that in their essential characteristics the protoplasm of these cells resembles that of other cells which we have better opportunities of examining chemically. With regard to the structure of the nuclei, there is nothing to add to what has already been said relating to cell nuclei generally.

On the other hand, various specialised varieties of epithelium differ considerably from ordinary protoplasm. The tegumentary epithelium loses near the surface its protoplasmic character, and the cells become filled with horny material or keratin; this is exaggerated in certain parts like the nails and hair. Other forms of epithelial growth become calcareous, as in the enamel of the teeth; and in the invertebrate sub-kingdoms, the exoskeletons and shells are found to be composed of chitin, spongin, conchiolin, and other forms of albuminoid material, more or less permeated with calcareous deposit; and in a few cases, as in the ascidians, a carbohydrate material akin to cellulose is secreted by the epidermal structures.

In the various forms of secreting epithelium there are many points of chemical interest to be noted. It will be more convenient to reserve a detailed study of each until we actually deal with the secretions themselves. At the same time this will afford us an opportunity of glancing at secretion as a whole, the formation of ferments within cells, and the precursors of ferments or zymogens.

In connection with nerve-epithelia, the only one which we shall discuss is the retina with its various pigments.

PAVEMENT EPITHELIUM (ENDOTHELIUM)

This form of epithelium, which consists of a single layer of flattened cells, fitting together like the stones in a mosaic pavement, is found lining the interior of the heart and vessels, and of the serous membranes. In the serous membranes, openings exist between the cells, and from these stomata, as they are termed, capillary lymphatic vessels lead. The pulmonary alveoli are lined by flattened epithelial cells, very like those found in the vascular system; from the point of view of embryology they are however different, being hypoblastic, while endothelium proper is mesoblastic. These cells are extensible and elastic, and (as is seen in the capillaries, the walls of which consist only of endothelium) contractile also.

The outlines between these cells can in all cases be rendered visible by staining with silver nitrate. The cement between the cells has the power of forming a compound with this salt, which is reduced by light, and minute granules of metallic silver are thus deposited in it, marking out in black or brown lines the contours of the cells. The cement substance of epithelium is thus similar in this particular to the ground substance of connective tissue, and doubtless both have a similar composition, consisting chiefly of mucin.

COLUMNAR EPITHELIUM

This consists of a single layer of elongated nucleated cells. Such an epithelium lines the alimentary canal from the cardiac orifice of the stomach downwards, and also most of the ducts of secreting glands. When the columnar cells are short, the term cubical epithelium is employed.

The border of columnar cells is more strongly refracting than the body of the cell, and though there are no differences in its resistance to reagents, it no doubt consists of somewhat modified protoplasm. The body of the cell is often vacuolated, and often contains numerous fat globules; this is especially the case in the columnar epithelium of the small intestine, during the absorption of fat. These fat globules can be identified by the deep black colour they give with osmic acid (*see* Absorption).

Columnar cells often break down to form goblet cells, and their more superficial protoplasm is transformed into mucin, the chief constituent of mucus.

CILIATED EPITHELIUM

Ciliated epithelial cells are usually columnar in shape; the cilia are protoplasmic tapering processes; in the human subject 4 to 8 μ in length, but in many invertebrates, like the mussel, they are much larger.

Ciliary movement is independent of the circulatory and nervous system, but it is dependent on nutritional changes occurring in the cell with which the cilia are connected, as all movement ceases when they are severed from the cell. The conditions most favourable to ciliary action are a temperature a little above that of the body (40° C.), and free access of oxygen.[1] The movement is retarded by cold, by heat a little over 40° C. (this coagulates the proteids of the protoplasm of which they are composed); weak acids and all strong chemical reagents also kill cilia. Carbonic acid, chloroform and ether stop ciliary action, but the cilia recover when the poisonous vapour is replaced by oxygen. Distilled water acts as a protoplasmic poison here as elsewhere.

If cilia are allowed to work after being removed from the body, they will in a varying time get languid and finally stop. If for instance a few bars of the gill of the sea mussel be mounted in a little sea water, and watched with the microscope, they will probably finally be brought to a standstill in about two hours. This is no doubt a condition resulting from fatigue; and fatigue in its turn is, as in muscular tissue, the result of the accumulation of the products of activity. In the case of muscle, sarcolactic acid appears to be a substance that especially tends to cause fatigue; probably in the case of cilia, an acid is also produced; at any rate a dilute alkali will set the cilia going again. A drop of dilute potash (1 part KHO to 1000 of water), passed under the cover-glass, will cause the cilia in the specimen just mentioned to work vigorously once more. If an acid is produced by the activity of the cilia, the potash no doubt neutralises this, and thus the activity of the cilia, which was hindered by the acid, is restored. The alkali may also act by increasing the amount of imbibition water (*see* p. 188).

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[1] Cilia will, however, like muscle, continue to work some time in an atmosphere containing no oxygen. Their protoplasm, like that of muscle, is able to store up oxygen for future use (Sharpey: *see* Quain's *Anat.* vol. ii. p. 58).

In certain particulars, ciliary action resembles amœboid action: it is for instance accelerated and hindered by the same reagents. On the other hand ciliary movement resembles muscular movement: it is not due to contractility occurring in all directions, but, as in muscular movement, in one direction only. Engelmann[1] has suggested that the contractile protoplasm is situated chiefly on the concave side of the curved cilium, so that on contraction the cilium will be brought downwards, and on the contractile motion ceasing, the cilium will be erected by the elastic recoil of the substance forming its convex border. Engelmann also states that cilia are doubly refracting.

Ciliated epithelium in man lines the greater part of the respiratory passages, the Fallopian tubes and uterus, some of the ducts of the testis (vasa efferentia and coni vasculosi), and the cerebrospinal canal. In some of the lower vertebrates, like the frog, the pharynx and œsophagus are lined by ciliated epithelium, and in the human subject there is an indication of the same state of things having existed, for the lining cells of the ducts of some of the minute glands opening into the pharynx retain the cilia which have been lost from the general surface of the mucous membrane. In most of the above cases, as the name mucous membrane suggests, many of the ciliated cells may, like columnar cells, break down into goblet cells with the formation of mucin.

MUCUS

Mucus is the name given to the slimy secretion of the surfaces of various internal cavities (alimentary canal, respiratory passages, bladder, uterus, &c.), and in certain lower animals, e.g. the snail, it is poured out on the external surface of the animal. The membranes that line these cavities are called mucous membranes.

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[1] Engelmann, article in Hermann's *Handbuch*, 1879.

In some cases, certain of the lining epithelium cells yield mucin, the chief constituent of the secretion, by the formation of what are called goblet cells (fig. 71). The more superficial part of the cell protoplasm undergoes certain changes, which result in the formation of a highly refracting globule of mucin ; the precursor of mucin within the cell is called mucinogen ; after the mucin is expelled, the basal portion of the cell alone remains. This may once more grow into a normal epithelial cell, and may again undergo this mucoid degeneration.

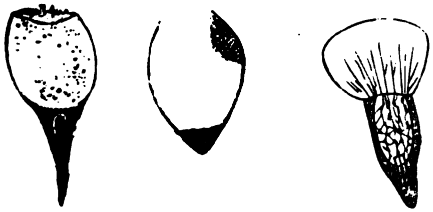

FIG. 71.—Goblet cells. Highly magnified (Klein).

In other cases the mucin is chiefly furnished by certain small racemose glands, situated beneath the general epithelial lining, with its duct opening on the surface. Here the cells of the acini of the gland undergo, as in the mucous salivary glands, the same transformation of the cell protoplasm into mucinogen, and this suspended in an alkaline liquid is expelled as mucin through the duct upon the surface of the mucous membrane.

The chief properties of mucin are its stickiness and viscosity and its solubility in dilute alkalis like lime water ; from these solutions it is readily precipitated by acetic acid, in excess of which it is insoluble. In composition, it consists of a globulin in combination with a carbohydrate called animal gum. By treatment with dilute sulphuric acid, the animal gum is converted into a sugar, which, like grape sugar, reduces alkaline solutions of cupric hydrate.

It is probable that there are several different kinds of mucin, i.e. different proteids combined with animal gum ; that obtained from the snail, for instance, is distinguished by Hammarsten [1] into foot mucin (obtained from the foot), and mantle mucin (obtained from the mantle) ; the properties of these two substances are slightly different from one another, and from the mucin obtained from saliva, mucus, &c. ; and these in turn differ from the mucin found in the ground substance of connective tissue. All mucins, however, are alike in the reactions that have been already mentioned, viz. their tenacity, their precipitability by acetic acid, and the fact that a reducing sugar is obtainable from them.[1] (Some more particulars concerning mucin will be found under the heading Connective Tissues, p. 476; *see* also p. 144.)

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[1] Hammarsten, *Pflüger's Archiv*, xxxvi. 373.

The pseudo-mucin of ovarian fluids differs from true mucin in not being precipitable by acetic acid; the same is the case with colloid material, formed in colloid degenerations of tumours, and contained within the vesicles of the thyroid body. Pseudomucin and colloid substance are probably identical (*see* p. 353).

Nucleo-albumins are like mucin in their physical characters, and in many of their reactions. The slimy material in bile was long mistaken for mucin; it, however, is not a compound of a proteid with a carbohydrate, but with the phosphorised substance known as nuclein. Such a nucleo-albumin we have already described as a constituent of the white blood corpuscles; similar substances occur in the other animal cells; Hammarsten,[2] for instance, has described one in the cells of the submaxillary gland (which contain however true mucin in addition).

Such considerations show that all slimy substances do not necessarily contain mucin; and it is especially the nucleo-albumins that must be carefully distinguished from that material.

The chief solid constituent of mucus, then, is mucin; epithelial cells, and débris of such cells, and a few leucocytes are also present, and these are suspended in a liquid which is doubtless a transudation from the blood; it has an alkaline reaction, and contains a certain small proportion of proteids and extractives, as well as mineral salts like those in the blood itself. The following table gives a few analyses that have been made of mucus.[3]

Parts per 1000	Tracheal Mucus (Wright)	Nasal Mucus (Berzelius)	Nasal Mucus (Nasse)
Water	956·0	933·7	955·6
Solids	44·0	66·3	44·4
Mucin	32·0	53·3	23·7
Other organic substances	4·0	10·4	9·8
Fats	—	—	2·8
Mineral Salts	5·0	5·6	8·1

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[1] These are the three characteristics of the mucin-group as defined by Hammarsten. *Chem. Centralbl.* 1884, p. 814.

[2] Hammarsten, *Zeit. physiol. Chem.* xii. 163.

[3] I am indebted to Charles's *Physiological and Pathological Chemistry*, p. 289, for this table.

The mucus of various parts differs a little in appearance and in reaction. Charles[1] describes the varieties as follows :--

Buccal mucus.	Transparent, viscid, alkaline.
Stomachal mucus.	Thready, greyish, alkaline.
Intestinal mucus.	Greyish, viscid, alkaline, rich in fatty particles, and suspended epithelium cells.
Vesical mucus.	Gives a cloudy appearance to urine.
Vaginal mucus.	Slightly viscid ; acid.
Cervical mucus (that of the neck of the uterus)	Slightly viscid, greyish, transparent, alkaline.

The amount of mucus normally secreted is small, merely sufficient to lubricate the surface ; in the case of the respiratory cavity, it entangles dust particles from the inspired air, and it together with this foreign matter is removed by the activity of the ciliated epithelium. It is stated that the mucus of the alimentary tract may aid digestion.

In cases of mild inflammation of the mucous membranes (catarrh), the amount of mucus secreted is increased. In more severe cases, the leucocytes become abundant, and the secretion is called muco-purulent, that is, a mixture of pus with mucus.

Sputum consists of the secretion of the mucous membrane of the respiratory tract mixed with a certain amount of saliva and occasionally nasal mucus. The following are some particulars concerning the different kinds of sputa in a few important diseases.

Quantity.--This is very variable, especially in bronchitis. In phthisis it may range from 80–150, in pneumonia from 26–300 grammes per diem.

Colour. In chronic inflammation of the bronchi it may be studded with black particles of carbon, especially in those living in a sooty atmosphere. In acute cases of bronchitis it is yellowish, owing to admixture with pus. In pneumonia the typical sputum is rusty, i.e. brown or yellowish-red, from the presence of altered blood pigment. As hepatisation proceeds, the sputum becomes greyish or purulent. In phthisis the expectoration may be tinged with bright blood.

Viscidity.--The most viscid expectoration is that of pneumonia. The most watery expectoration occurs in the early and late stages of bronchitis.

Odour.--In bronchiectasis and gangrene of the lung, the sputum has a putrid odour.

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[1] Charles, *loc. cit.* p. 288.

Quantitative Analyses (Percentages)

Disease	Water	Solids	Organic Matters	Mucin	Proteids	Fat	Extractives	Ash	Remarks
Bronchitis	97·6–98·3	1·7–2·3	1·17–1·7	0·69–1·2	—	—	0·08	0·53–0·64	-
Pneumonia	90·09–93·6	6·3–9·01	5·5–8·35	1·1–1·39	3·09	0·02–0·32	2·8–3·95	0·66–0·77	Fibrin present in sputum. Sputum very rich in NaCl, increasing as hepatisation proceeds.[1] Note high percentage of proteids and extractives.
Phthisis	94·5	5·5	4·7	1·8–2·4	0·29–0·39	0·36–0·39	1·6–2·01	0·76–0·8	Often contains tissue elements of lungs, of which elastic fibres are most easily recognised. It also contains tubercle bacilli.

SECRETING EPITHELIUM

Epithelium is a tissue which exhibits varying degrees of vitality in different parts according to its function ; thus the outer portions of the epidermis are almost entirely non-protoplasmic, and they undergo few physical and chemical changes of any kind ; their function is simply protective. In the secreting glands, on the other hand, the epithelial cells are composed of protoplasm which is the seat of the most active and remarkable chemical operations, the building up of new substances which are discharged as a secretion to fulfil important functions elsewhere ; or the substances may be simply taken from the circulating fluid by the cells, and poured out from them to form an excretion: that is, these substances are simply got rid of and discharged from the body by this means.

A secreting epithelium may be considered as a partition between the blood, or, more properly speaking, the lymph, on the one side, and the lumen of the secreting gland on the other. From the lymph the materials are taken by the secreting cells and then worked up into the components of the secretion, and finally discharged on the other side into the lumen, and thence by the ducts of the secreting gland to their destination.

A useful contrast is drawn by Dr. McKendrick[2] between the activities of three important varieties of organs :—

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[1] The amount of chlorides in the urine is correspondingly low.

[2] *Physiology*, vol. i. pp. 484–5.

(1) **Muscles.** (2) **Electrical organs.** (3) **Secreting cells.**

If *a* be contraction, *b* electromotive phenomena, and *c* metabolic or chemical changes : in a muscle *a* is large, *b* and *c* relatively small ; in an electrical organ, *a* is apparently absent, *b* is large, and *c* relatively small ; and in secreting cells *a* does not occur as an active contraction, though the cell may slowly change in form and bulk, *b* occurs, but is comparatively small, while *c* is relatively large. Thus the differences between these three varieties of protoplasm are very largely differences of degree only. A further resemblance to be briefly noted is, that just as muscles are supplied with nerves along which motor impulses are conveyed, and electrical organs with special nerves, excitation of which causes activity of the organs they supply, so secreting cells are in many cases at least supplied with nerves, excitation of which causes the activity of the gland cells they supply ; and in some cases where a gland receives two nerves with different functions, excitation of one will produce a secretion differing somewhat from that produced by exciting the other.

The amount of secretion is in some cases, as in that of the kidney, very largely influenced by the amount of blood reaching the organ, and by the blood pressure ; this again is dependent on the size of the blood vessels, which is regulated by the vaso-motor nerves that supply their muscular tissue.

In most cases the secreting cells that line the acini of a secreting gland are large spheroidal or polyhedral cells. In other cases, as in the convoluted tubules of the kidney of some animals (possibly the epithelium of the cerebro-spinal cavity must be included here), the cells are ciliated.

No histological differences can be made out in certain glands according to the secreting activity of the cells ; instances of such glands are the kidneys and the sweat glands ; these are glands that are excretory in function, they form no special ferment for use elsewhere, and their activity depends very largely on the amount of blood passing to them.

There are, however, certain other cases in which a distinct difference between the active and resting condition of the secreting cells can be made out. We have, in fact, already studied the changes in one instance, namely, the formation of mucin in ciliated and columnar cells. A similar transformation of protoplasm into mucin (or mucinogen, as it is called while still within the cells) is seen in the acini of the numerous little racemose mucous glands of the mouth, pharynx, trachea, and œsophagus, as well as in the cells of the mucous alveoli of the submaxillary and sublingual salivary glands. The cells distended with granules of mucinogen disintegrate, extruding mucin, and their place is then taken by protoplasmic cells which were formerly pressed against the basement membrane by the swollen mucigenous cells, forming the darkly staining demilunes. These protoplasmic cells undergo in time the same mucoid degeneration.

In the case of the sebaceous glands and the mammary gland, the secreting cells become swollen with fat-globules; it is, in fact, a fatty degeneration of the protoplasm, the cells disintegrate, and the secretion is filled with the minute fat-globules thus liberated. In the milk secreted during the first few days after lactation commences, some of the secreting cells filled with fat-globules may be readily discovered, but later these colostrum corpuscles, as they are termed, are not found, the disintegration of the cells taking place entirely in the alveoli of the mammary gland. In other cases still, secretion does not involve either complete or partial destruction of the secreting cells themselves, but nevertheless the changes occurring during secretion are distinctly visible to the microscope. As instances, the parotid gland, the pancreas, and the central cells of the gastric glands may be mentioned. In the inactive condition of these glands, the cells are seen to be packed full of distinct granules which obscure their nuclei. The granules are imbedded in the protoplasm of the cells, and the latter almost completely fill the alveoli, scarcely any lumen being perceptible (fig. 72 A). After a short period of activity the granules (which may be seen either in the perfectly fresh condition of the gland, or by staining with osmic acid) disappear from the outer part of the cells, the inner part being distinctly granular, and some granules being apparently free within the lumen of the alveolus which is now becoming distinct (fig. 72 B). With more prolonged activity, such as is produced by a dose of pilocarpine, the clear outer zone increases in extent, and the granules are found only at the free border of the cells (fig. 72 C). The nuclei become distinct and the cells smaller; they now, moreover, being composed chiefly of non-granular protoplasm, stain readily with carmine. According to

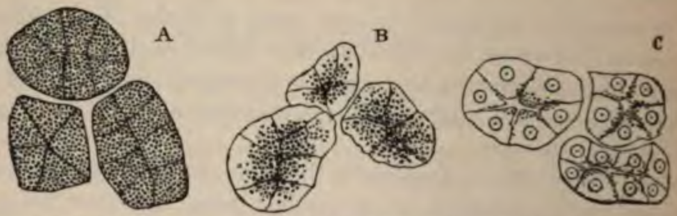

FIG. 72.—Alveoli of Serous Gland. A, at rest; B, after a short period of activity; C, after a prolonged period of activity. (Langley.)

Heidenhain[1] and Langley[2] the three processes—growth of the clear protoplasm, conversion into granules, and discharge of these into the lumen—are all proceeding simultaneously in different parts of the cell during activity. The material which thus accumulates within the cell is not, however, the same as that which appears in the secretion; the most important constituent of the secretion is the zyme or ferment, and the granules are either composed of, or indicate the presence of, a zymogen, or ferment precursor. The zymogen differs somewhat in its properties from the ferment, but is converted into the ferment with great readiness; pepsinogen in the stomach cells, and trypsinogen in the pancreatic cells, may be mentioned as instances of zymogens.

In the case of the pancreas it has, however, been shown that the granules which have been usually identified with the zymogen may occur in the absence of zymogen. In animals from whom food has been withheld for longer than twenty-four hours, glycerine fails to extract any ferment from the pancreas; but microscopic examination shows that the cells are still filled with granules. The conclusion must therefore be drawn either that these granules are something different from those ordinarily seen, or that the granules are not themselves the zymogen, but only the carriers of it. Under ordinary circumstances, however, the presence of granules indicates the presence of the zymogen; and zymogen without granules has never been observed (Levascheff[3]).

In most cases the secretion when formed passes into the cavity lined by the secreting epithelium. In some cases, however, as in the liver (and the same has been seen after forcible injection in the pancreas also), the secretion collects in vacuoles in the secreting cells from which it passes into the smallest ducts by means of intracellular canaliculi.[4]

COMPOUND EPITHELIA

In the case of the transitional epithelium of the bladder and ureters, the stratified epithelium of the cornea and œsophagus, the cells are apparently composed of simple protoplasm with nuclei. But in the stratified epithelium which forms the epidermis, and to a less extent in that lining the interior of the mouth, the surface layers of cells (those which form the horny layer) have their protoplasm replaced by keratin or horny material. In the nails, horns, and hoofs, this conversion into keratin is still more marked, and in addition there is a deposit of calcareous salts, especially calcium phosphate. In hairs and feathers also the chief organic constituent is keratin ; cells filled with fat or pigment granules may occur in the medullary portions of the hair. In both hair and feathers silica has been described as a constant and important mineral constituent (27–40 per cent. of the ash ; von Bibra) ; iron may also occur.

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[1] *Studien d. phys. Inst. zu Breslau*, 1868.

[2] *Journ. of Physiology*, 1879; *Phil. Trans.* 1881.

[3] Levascheff worked under Heidenhain's superintendence. *Pflüger's Archiv*, xxxvii. 82.

[4] Quain's *Anatomy*, 632, 638.

The deeper portions of stratified epithelia, which become horny in their surface layers, remain protoplasmic ; in the skin the protoplasmic layers (Malpighian layer) and the horny layers proper are separated by two thin layers, the stratum granulosum and the stratum lucidum. The granules in the former layer are composed of a substance which stains deeply with carmine (Langerhans[1]). It is termed *eleidin*, and is supposed to be an intermediate condition in the replacement of protoplasm by keratin. In the cells of the Malpighian layer, granules of a dark pigment called *melanin* are found ; these are especially abundant in the skin of negroes.[2] The cells of the epidermis have a small amount of cementing substance between them, which, like the ground substance of connective tissue, dissolves in weak alkalis ; and by such treatment the cells may be separated from one another.

Keratin

Keratin or horny material belongs to the class of substances that are called *albuminoids*. It is exceedingly insoluble, and can be freed from other substances by treating cuticle, hair, hoofs, nails, &c. with ether, alcohol, water, and dilute acids. A very similar substance called neurokeratin can be obtained from nervous structures, these being, like the epidermis, epiblastic in origin.

It is not affected by boiling with water ; but when heated with water in closed vessels to 150°–200° C. it forms a turbid solution. It is not affected by weak acids in the cold, but is dissolved by boiling glacial acetic acid ; it is decomposed by boiling with mineral acids, yielding with sulphuric acid products very similar to those obtained from proteids, viz. leucine, tyrosine, aspartic acid, and volatile fatty acids. Like proteids also it gives off when burnt the same peculiar odour.

Elementary analyses, from their close resemblance one to another, seem to point to the fact that keratin is a chemical unit, but as in the case of proteids we are not acquainted with its rational formula. The following five analyses[1] show, however, discrepancies in the percentage of sulphur present; there is a remarkably large percentage of sulphur, and most of it is very loosely combined;[2] no doubt the different methods adopted for the estimation of the sulphur employed by the various investigators will sufficiently account for the different results obtained, sulphur being a difficult substance to estimate correctly when occurring in organic compounds.

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[1] *Archiv f. mikr. Anat.* 1873.

[2] The dark pigment deposited in the skin in cases of Addison's disease is apparently of the same nature.

	Keratin from Hair V. Laer	Nails Mulder	Horn Tilanus	Hoof Mulder	Hair Kühne and Chittenden[3]
C	50·60	51·00	51·03	51·41	49·45
H	6·36	6·94	6·80	6·96	6·52
N	17·14	17·51	16·24	17·46	16·81
O	20·85	21·75	22·51	19·49	23·20
S	5·00	2·80	3·42	4·23	4·02

Melanin

The term melanin has been applied to a large number of black pigments occurring in the body; thus we have already noted a black pigment in the blood of persons affected by malaria and other diseases (Melanæmia, *see* p. 310). This, no doubt, is derived from hæmoglobin; and perhaps the other black pigments of the body occurring in the retina, in the skin, and in melanotic sarcomata may ultimately have the same origin. There is no doubt that these pigments are, however, not all identical; elementary analyses show this; for instance, iron is present in some, absent in others. In the tissues of the lungs and bronchial glands the black pigment that occurs there simply consists of particles of carbon breathed in with the atmospheric air.

The black pigment of the skin and of the hair has been examined by Sieber, who made some few percentage estimations of the elementary composition of the substance, but obtained very discordant results. The elements present are carbon, nitrogen, hydrogen, and oxygen.[4] It has never been crystallised; it is soluble in water, alcohol, ether, acids, and strong alkalis; the brown solution produced by dissolving it in hot potash is decolorised by chlorine water.

(The subject of melanin will be more fully dealt with under the Retina (p. 457) and Melanotic Sarcoma, chap. xxiii.).

[1] The first four analyses are quoted from Hoppe-Seyler's *Physiol. Chem.* p. 90.

[2] Hoppe-Seyler, *Physiol. Chem.* p. 91.

[3] *Zeit. Biol.* xxvi. 291.

[4] *See* also Hodgkinson and Sorby, *Journ. Chem. Soc.* 1877, p. 427.

Turacin

This is the only one of the many pigments in birds' plumage that has been satisfactorily examined. It is obtained from the touracou, or plantain-eater, of the Gambia. It is of a crimson colour; it is not crystalline; it shows two absorption bands between D and E, and is remarkable as being one of the few animal compounds that contain copper (Church[1]).

Skeletins

The term skeletin[2] has been applied to a number of nitrogenous but sulphur-free substances found in the skeletal tissues of invertebrates. They appear to be intermediate between carbohydrates and proteids, and Krukenberg considers that they are amido-derivatives of carbohydrates. The skeletal tissues of invertebrate animals are, as a rule, epidermal (not mesodermal, as in vertebrates). The skeletins appear to take the place of keratin in invertebrate animals. The principal skeletins are chitin, conchiolin, cornein, spongin, and fibroin.

Chitin.—This substance has a very wide distribution among the invertebrate groups. It is in the arthropoda that it is found to the greatest extent; it forms the membrane of the ovum, the cuticle of the adult, with its appendages, the supporting substance in the tracheæ of insects, &c. It is also found in mollusca (jaws and odontophore); in worms (e.g. the setæ of annelids). It forms the membrane of the ova in other groups, and the cyst-wall in encysted forms of protozoa; but its presence in these and a few other situations where it has been described has not been fully proved.[3]

Chitin is frequently impregnated with mineral salts, calcareous salts in the crustacea, silica in the lingual ribbon of certain molluscs.

Preparation.—Chitin may be prepared in large quantities from the shell of the crab or other crustaceans, and in smaller quantities from the wing-cases of the cockroach or other insects; in the case of the crab and lobster calcareous salts must be first dissolved out by hydrochloric acid; this operation is not necessary if insects' or beetles' wings be used; the substance is boiled with solution of caustic soda; this leaves the chitin undissolved: the residue is then dissolved in

[1] *Phil. Trans.* vol. clix. (1870), p. 627. A number of observations on the pigments of birds' feathers will be found in Krukenberg's *Vergleich. Physiol. Studien.*

[2] Krukenberg, *Zeit. Biol.* xxii. 241.

[3] Chitin is not wholly epiblastic, however; it is found, for instance, in mesoblastic structures, e.g. the cartilages of sepia and limulus (*see* Invertebrate Cartilage, chap. xxii.). In those animals which possess chitin instead of keratin, the neurokeratin of the nerves is replaced by neurochitin.

concentrated hydrochloric acid, and precipitated from this solution by excess of water. This is once more dissolved in acid and reprecipitated by water, and to obtain the substance in a state of purity the operation should be again repeated.

Properties.—Chitin is thus, like keratin, a very insoluble substance, but differs from keratin in its insolubility in concentrated solutions of the caustic alkalis. Concentrated mineral acids will alone dissolve it. It is colourless and amorphous; it has the formula $C_{15}H_{26}N_2O_{10}$; and when heated with concentrated mineral acids it yields not a fermentable sugar as Berthelot[1] supposed, but a nitrogenous substance, glucosamine, which reduces alkaline solutions of cupric salts as sugar does (Ledderhose[2]). The equation which represents the reaction is as follows:—

$$\underset{\text{[chitin]}}{2C_{15}H_{26}N_2O_{10}} + 2H_2O = \underset{\text{[glucosamine]}}{4C_6H_{13}NO_5} + \underset{\text{[acetic acid]}}{3C_2H_4O_2}$$

Glucosamine is an amido-derivative of dextrose; if a molecule of hydroxyl (HO) in dextrose be replaced by one of NH_2, the formula of glucosamine will be obtained. If chitin be boiled with concentrated hydrochloric acid for about an hour, the solution becomes black or brown, and on evaporation rhombic crystals of the hydrochloride of glucosamine crystallise out. The formation of these crystals is a very convenient test for chitin. On a small scale the crystallisation can be carried out on a microscope slide, but on a large scale crystals an inch or more in length are obtainable; these are brownish at first, but after two or three recrystallisations may be obtained perfectly colourless. It is dextro-rotatory; $a_D = +70{\cdot}6°$ (Gamgee[3]). The sulphate of glucosamine may be obtained by treating chitin with sulphuric acid; by acting on this with barium hydrate the pure base which is also crystalline may be obtained. By the action of hydrochloric acid on chitin, not only is the hydrochloride of glucosamine formed, but also a dextrin-like carbohydrate (Krukenberg[4]).

Conchiolin.—This is the skeletin that forms the organic bases of the shells of gastropods. The shells are first macerated in hydrochloric acid, and the residue boiled with caustic soda. Like chitin, conchiolin remains undissolved. Its formula is $C_{30}H_{48}N_9O_{11}$. Like chitin, it dissolves in hot concentrated mineral acids. It does not give the xanthoproteic, Millon's, nor the Adamkiewicz colour reactions. On decomposition it yields leucine, a small and doubtful quantity of glycocine, but no tyrosine, and no glucosamine. Its formula and its decomposition products clearly distinguish chitin from conchiolin.

The cement substance between the eggs of various molluscs, whose shells and egg-capsules contain conchiolin, is coloured red by heating with Millon's reagent, and contains a substance allied to keratin (Krukenberg, *loc. cit.*).

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[1] *Comptes rendus*, xlvii. 227.

[2] *Zeit. physiol. Chem.* ii. 213. *Ibid.* iv. 139.

[3] *Physiol. Chem.* p. 301.

[4] *Zeit. Biol.* xxii. 480.

Cornein.—This is the skeletin originally described by Valenciennes and Frémy in certain corals (Gorgonia, Antipathes, &c.). It has been more fully examined by Krukenberg.[1] Its formula is $C_{30}H_{44}N_9O_{13}$. It is thus nearly allied to conchiolin. On decomposition it yields leucine and glycocine. It gives, moreover, a red colour with Millon's reagent. The mineral matter in corals is chiefly calcium carbonate.

Spongin, the skeletin of sponges, like the two substances just described, yields on decomposition leucine and glycocine.[2] It gives none of the three colour reactions just enumerated; and, like conchiolin, yields on digestion peptone-like substances, which differ from true peptones and albumoses by not giving the colour reactions in question: thus they both differ from keratin, which is not digestible. The mineral matter in many sponges consists of siliceous spicules.

Fibroin, the substance of which spiders' webs are composed, is nearly allied to these substances. It is an insoluble substance, except in concentrated mineral acids and alkalis. It yields on decomposition leucine, tyrosine, and glycocine and gives all the colour reactions just enumerated like a proteid.

Silk is a very nearly allied substance, but is considered by Weyl[3] to be a true proteid. Its percentage of nitrogen is lower than that of fibroin.

Tunicin

Cellulose is found in very few places in the animal kingdom; it composes the tunic of the ascidians or tunicates, and it is there called tunicin. It is also found in the zoocytium or mucilaginous investing matrix of the *Ophrydium versatile*,[4] and perhaps in some allied protozoa. In the case of the Ophrydium it is interesting to note that chlorophyll, another vegetable product, is present also.

Animal cellulose $(C_6H_{10}O_5)_n$ may be purified by washing with water, dilute hydrochloric acid, dilute caustic potash, alcohol, and ether. By the prolonged action of sulphuric acid it is converted into a fermentable sugar which reduces Fehling's solution. According to Berthelot,[5] this change is not effected in tunicin until after some weeks' boiling with the acid. Tunicin gives a blue colour, the cellulose from Ophrydium a brown colour with iodine and sulphuric acid.

THE RETINA

This is the innermost lining of the eye, and consists of both nervous and epithelial structures. The nerve-fibres passing through the various complex innermost layers terminate in a nerve-epithelium, which is called the layer of rods and cones; and it has been satisfactorily proved that it is this layer upon which the images of external objects are focussed by the refractive apparatus in front of it. The impressions of light affect the rods and cones, and thence they are propagated as nervous impulses *via* the optic nerve to the brain. External to the layer of the rods and cones is a layer of hexagonal epithelium cells containing a black pigment. It is these two layers that we have to describe in detail.

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[1] *Berichte d. chem. Gesellsch. Berlin*, xvii. p. 1843.

[2] Zalocostas (*Compt. rend.* cvii. 252) found also traces of tyrosine, butalamine, and glycalanine ($C_5H_{12}N_2O_4$). Its constitution resembles that found in proteids by Schützenberger (*see* p. 115).

[3] Weyl, *Ber. d. chem. Gesellsch. Berlin*, xxi. 1407, 1529.

[4] Halliburton, *Quart. Journ. Mic. Science*, July 1885.

[5] Berthelot, *Ann. de chimie et de phys.* série 3, tome lvi. p. 153.

The retina as a whole gives indications of its twofold structure, nervous and epithelial. Its reaction is stated to be acid; and, like most animal tissues, it becomes opaque after death. Water dissolves out from it proteids, gelatin, and mucin, the two last-named substances being probably derived from the supporting connective tissue it contains. Alcohol dissolves lecithin from its nerve-fibres and cells. Other reagents are employed to dissolve out other constituents, such as the pigments, from the rods and cones. Cahn[1] gives the following quantitative results:—

Water	86— 89	per cent.
Solids	14— 11	„
Proteids[2]	4— 6	„
Gelatin	1·3—1·7	„
Cholesterin	0·3—0·8	„
Lecithin	1·0—2·9	„
Fat	0·05—0·5	„
Salts	0·7—1·2	„

Most of our knowledge of the chemistry of the retina is the result of the labours of Kühne and his pupils. A *résumé* of the chief facts will be found in Kühne's article in Herrmann's 'Handbuch der Physiologie' (1879), vol. i. p. 235.

The Hexagonal Pigment Cells of the Retina

The pigmentary layer of the retina was at one time supposed to be a part of the choroid or vascular coat of the eye, but the facts of embryology have shown that it is in reality part of the retina, and is developed like the rods and cones from the epiblast, whereas the choroid is developed from mesoblastic tissue. The choroid, however, contains branched cells in which is pigment identical with the black pigment of the retina.

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[1] Hoppe-Seyler's *Physiol. Chem.* p. 699.

[2] Three in number—one resembling myosin, coagulating at 55° C., another like mucin, and a third like serum-albumin.

The cells are flattened, six-sided, and form a pavement covering the outer portions of the rods and cones, and sending down long processes between them.

Externally the cells consist of a layer of neurokeratin; internally they are protoplasmic; in the protoplasm are found one or two nuclei and large numbers of black rod-shaped pigment granules. Deposits of a substance called myeloidin by Kühne, and in some animals of yellow fat-globules, are also found.

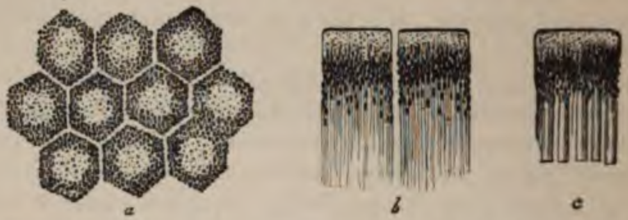

FIG. 73.—Pigmented Epithelium of the Human Retina (Max Schultze) highly magnified. *a*, Cells seen from the outer surface. *b*, Two cells in profile with fine offsets extending inwards. *c*, A cell still in connection with the outer ends of the rods.

The black pigment.—Fuscin.—Owing to movements in the cell protoplasm of the nature of amœboid movements, the granules of black pigment are differently distributed at different times; after keeping a frog for several hours in darkness, the pigment will be found in the cell bodies, and in the parts of the processes nearest to the cell bodies. But if the frog has been exposed for a similar time to sunlight before death, the pigment granules will be distributed chiefly along the processes, and a relatively small number remain in the bodies of the cells themselves. In some animals (dog, cat, &c.) much of the retinal epithelium contains no fuscin, but the cells are filled with fine crystals (Max Schultze); this forms the *tapetum*. In some fish, e.g. bream, the tapetum (or pseudo-tapetum) contains guanine, a highly refracting substance; while in the ox and sheep the tapetum is merely fibrous tissue (Kühne and Sewall[1]).

Fuscin is one of the group of black pigments termed melanins. It has been investigated by Berzelius, who found it contained a small quantity of iron, by Scherer, who found no iron, and also by Rosow and Sieber. The percentage composition obtained by the various observers shows great discrepancies, and this, taking also into account their methods of preparing the pigment, renders it probable that they were not dealing with a pure substance. The failure of some observers, for instance, to obtain evidence of the presence of iron was due as Mörner[1] points out to their having used hydrochloric acid at one stage or other of their operations; this acid dissolves out nine-tenths of the iron from the pigment. May's method[2] of preparing fuscin is to boil several hundreds of retinæ in alcohol, then in ether, lastly in water; the residue is subjected to tryptic digestion for twenty-four hours; the pigment, nuclein, and neurokeratin remain undigested; the first-named impurity is dissolved by trituration with alkali, and the last-named must be picked out as well as possible with forceps.

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[1] *Verhandl. der naturhist. Vereins Heidelberg*, N.S. ii. Heft v.

Fuscin dissolves by boiling it a long time with concentrated sulphuric acid, or concentrated caustic alkalis.

Like all the other retinal pigments, fuscin is bleached in the air, only very slowly indeed. This is probably due to oxidation. The physiological relation of the fuscin-bearing cells with the rods and cones will be dealt with in the consideration of those structures.

There is considerable doubt as to whether this pigment is ultimately derived from hæmoglobin; Krukenberg considers it is more closely related to the lipochromes or fatty pigments. It is, however, undoubtedly nitrogenous. It does not belong to the group of brown pigments, many of which occur in plants called humous substances by Hoppe-Seyler,[3] since on fusing with alkali it yields no pyrocatechin or protocatechnic acid (Hirschfeld[4]). (*See* p. 149.)

Myeloidin.—Myeloidin, or myeloid substance, is not a chemical unit. The term is used as indicating that the cells contain a substance similar to that which forms the white substance of Schwann in nerve-fibres. It is also found in the rods, and will be there more fully dealt with.

Yellow fat-globules.—These are not present in all animals; they are especially abundant in the retina of the frog. The pigment can be extracted by ether, carbon bisulphide, benzene, &c. It shows two absorption bands between F and G. The yellow pigment was called *lipochrin* by Kühne. It is, however, exceedingly probable that this is the same pigment found generally in adipose tissue; it belongs to the class of pigments called lipochromes or luteins, and like all these pigments is slowly bleached by sunlight.

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[1] K. A. H. Mörner, *Zeit. physiol. Chem.* xi. 66–140. In this paper the references to the writings of the observers mentioned above will be found.

[2] *Untersuchungen aus d. physiol. Inst. der Univ. Heidelberg*, ii. 324.

[3] *Zeit. physiol. Chem.* xiii. 66.

[4] *Ibid.* xiii. 407.

The Rods and Cones

The rods and cones form the nerve-epithelium which receives the impressions of light from without. The accompanying figure shows the general shape and relative size of a rod and a cone. Each consists of two distinct segments, an inner and an outer. The outer or narrower segment is doubly refracting, and is stained darkly by osmic acid, while the inner segment is singly refracting, and stains as protoplasm does with carmine, magenta, &c.; the outer part of the inner segment is longitudinally fibrillated, while its inner part is granular or homogeneous; and in the case of the cones has been shown to undergo movements (Engelmann). The prolongation inwards of the rods and cones ultimately join the terminal nerve-fibrils of the optic nerve; the connection occurs in the outer molecular layer (Gunn[1]). The outer segments of the rods contain the pigment known as the visual purple or rhodopsin; the inner segments of the cones in birds, reptiles, and amphibia contain coloured oil-globules, known as chromophanes.

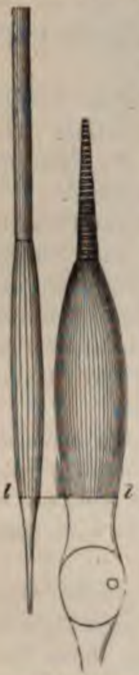

FIG. 74.—A Rod and a Cone from the Human Retina (Max Schultze) highly magnified.

The following animals have no cones: the ray, shark, sturgeon, bat, hedgehog, and mole. The following have no rods: lizards, serpents, tortoises, and perhaps all reptiles. Mammals have more rods than cones, except at the part where vision is most acute, viz. the macula lutea; here cones only are found. Birds have more cones than rods; the owl is an exception to this rule.

The outer segment of a rod can by the action of certain reagents, such as free toluylenediamine or its neutral acetate, be split into a number of cross discs; the indication of such a division can be seen in the fresh rod in the shape of indistinct transverse markings or groovings. Each disc so obtained retains its reddish purple tinge (due to rhodopsin), and is seen to consist of an outer ring of more solid material, filled with a less solid substance. The outer ring is composed of neurokeratin, the inner substance is what stains readily with osmic acid, namely, the myeloidin of Kühne. Myeloidin is not lecithin, as some have supposed; lecithin is not coloured nearly so darkly by osmic acid, nor is the black coloration removed by hydrogen peroxide, as it is in the case of myeloidin (Unna). Myeloidin is probably a compound or mixture of lecithin and a globulin (vitellin). The myeloidin can be dissolved out by concentrated solutions of certain neutral salts like ammonium chloride (Dreser[1]).

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[1] *Journal of Anat. and Physiol.* 1877.

The outer segment of a cone is smaller than that of a rod; the transverse markings are more distinct than in the case of the rods, but the separation into discs does not take place so readily; this is owing, as some have supposed, to the existence of a delicate membrane covering the entire outer segment. The outer segment of the cones contains no visual purple. It consists, however, as that of the rod does, of neurokeratin externally, myeloidin within.

Visual purple or rhodopsin.—Although H. Muller[2] in 1851, and Leidig[3] in 1857, noticed the red colour of the retina of the frog, it was not till twenty years later that Boll[4] discovered that the red colour of the living retina disappears under the bleaching influence of light, and that it is restored by darkness, disappearing, however, for good on the death of the animal. The subject was taken up by Kuhne,[5] who found he was able to study the reactions and properties of the substance which give the colour to the retina if observations on it are made in a chamber illuminated only by the sodium flame, yellow light having only a slight bleaching action on the pigment, which he called Sehpurpur (visual purple). It was first found that the pigment was contained only in the outer segments of most of the rods; it was completely absent in the cones, in the rods in the neighbourhood of the macula lutea, and in the rods near the ora serrata, the anterior border of the retina.

A solution of visual purple can be obtained by means of a 2–5 per cent. solution of the bile salts of the ox. The solution so obtained contains also a proteid resembling myosin (Dreser[6]). Such a solution can be best obtained from frog's retinæ, as it is easy to free these from blood. When evaporated to dryness *in vacuo*, an amorphous carmine-like powder is obtained on which light has very little action; this redissolves readily in a solution of bile salts, and when placed in a dialyser the pigment does not pass through the membrane.

On exposure to light, the visual purple first becomes yellow (visual yellow) and then colourless. The spectroscopic appearance of visual purple and visual yellow is shown in fig. 75, spectra 1 and 2; there are no well-defined bands, but a general absorption of the central regions of the spectrum. White light bleaches rhodopsin most quickly, then follows green, blue, and, after an interval, yellow, violet, orange, and red. The sodium flame takes about two hours to bleach a frog's retina, but is more convenient than a red flame, as by light of a red colour it is difficult to detect and avoid blood stains. The intermediate stage of visual yellow is bleached more quickly by rays from the violet end of the spectrum, or it may be that less yellow is produced under the influence of such rays.

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[1] *Zeit. für Biologie*, xxii. 23.

[2] *Zeit. f. wissensch. Zool.* iii. 234.

[3] *Lehrbuch d. Histologie*, p. 238.

[4] *Monatsber. d. Berlin Akad.* 12 Nov. 1876.

[5] Kühne, Ewald, Ayres, Mays, and others of Kühne's pupils, published numerous papers on the subject in the *Untersuch. aus d. physiol. Inst. zu Heidelberg*, vols. i. and ii.

[6] *Loc. cit.*

The rapidity with which visual purple fades increases with the temperature up to 76° C., at which temperature it disappears instantly even in the dark.

Alcohol, ether and chloroform, caustic alkalis and acids destroy the pigment. Putrefaction and tryptic digestion do not. Oxidising agents, such as ozone, hydrogen peroxide, osmic acid (the black colour produced with myeloidin having first been destroyed by hydrogen peroxide, or the myeloidin may be previously removed by ammonium chloride), ferric chloride, potassium chlorate, and iodate have no effect. These reactions show that visual purple is a substance already highly oxidised.

Such reactions, however, are of little interest compared to those produced by the action of light. That the bleaching action of light occurs during life was most conclusively shown by those experiments in which Kühne succeeded in obtaining what may be compared to photographic impressions upon the retina; these were obtained in rabbits. The animal was first put in darkness by covering its head with a black cloth, it was then exposed to the light of a window, and immediately decapitated, the eyes removed, and the retinal colours fixed by a solution of alum; a small bleached area corresponding in shape to the window, and about a millimetre square, was found on the retina next day. Such *optograms* may be preserved a long time by drying the retina *in vacuo* after removal from the alum solution.

Regeneration of visual purple.—This is continually taking place during life, and occurs especially in the dark. This phenomenon appears to be associated with the hexagonal pigment cells which send down their processes between the outer segments of the rods; if a piece of a fresh retina be lifted from the black pigment cells, and then be exposed to the light, it will become bleached, and if then the retina be placed in darkness the colour will not return as it does in the rest of the retina; but if the flap be replaced so as to touch the hexagonal cells, regeneration of the purple occurs. This function of the hexagonal cells does not seem to depend on the amount of fuscin they contain. It is possible that the rods contain the precursor of visual purple, and this is acted upon by some other substance from the hexagonal cells; or it may be that the hexagonal cells withdraw the supposed substance from the rods and work it up into visual purple.

The subcutaneous injection of pilocarpine causes in the frog (not in the rabbit) a hastening in the regeneration of the visual purple (Dreser).

The physiological uses of visual purple.—The rays of light which are focussed on the rods and cones produce in those structures certain obscure chemical changes which no doubt are very similar to those produced by the action of light upon a sensitive photographic plate. The most tempting hypothesis suggested by the discovery of visual purple was, that that substance is itself the sensitive chemical material, the changes in which are indicated by the changes of colour it

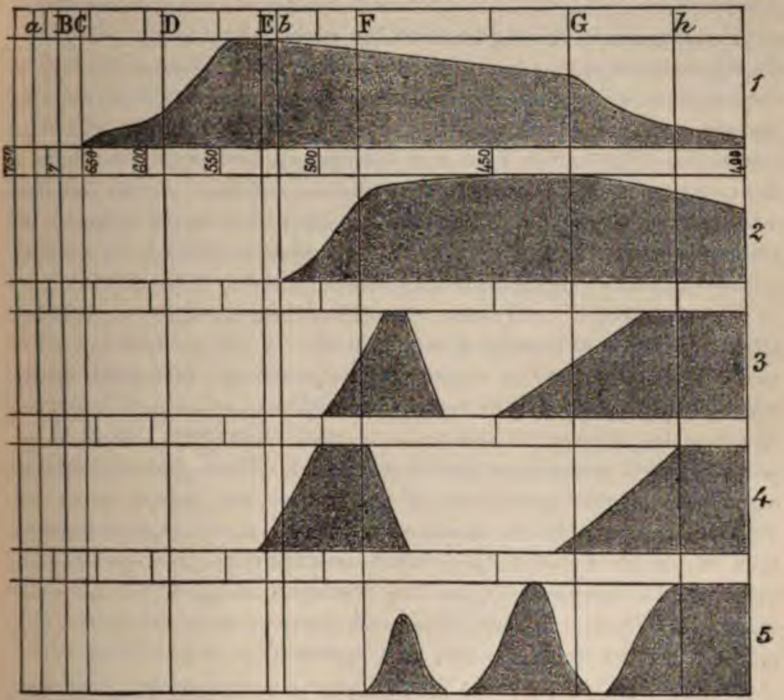

Fig. 75.—Diagrams of Absorption Spectra: 1, of visual purple; 2, of visual yellow; 3, of xanthophane in ether; 4, of rhodophane in turpentine; 5, of chlorophane in ether. This method of representing absorption spectra has been explained in connection with fig. 58, p. 276.

undergoes. But further research has shown that this view cannot be adopted, and that probably the changes in the visual purple are merely accidental accompaniments of other chemical changes that are as yet undiscovered. This conclusion is derived from the consideration that vision occurs in the absence of visual purple altogether; in birds and reptiles, for instance, it is absent, and in man the part of the eye which is most sensitive to light—the fovea centralis—contains no rods, and therefore no visual purple. It is altogether absent in the bat; but, on the other hand, it is present in the owl. Both these animals are nocturnal, so the habits of the bat will not explain its exceptional condition.

Chromophanes, the pigments of the cones.—In birds, reptiles, and fishes, the inner segment of the cones contains a coloured oil-globule, the colour varying greatly. By using large numbers of birds' eyes Kühne and Ayres [1] succeeded in preparing these coloured fats in large quantities. The retinæ were first dehydrated by absolute alcohol and then extracted with ether; the ethereal solution of the fat was evaporated to dryness and the residue treated with caustic alkali; the coloured soaps so formed were freed from excess of alkali by washing with water, and were separated by means of their different solubilities in various reagents; petroleum ether dissolving out a green substance, ether a yellow, and benzene a red material. It was not found possible to obtain the pigments in a state of purity, nor in a crystalline form; when the pigments were in association with fats or fatty acids instead of soaps they showed no difference in their solubility; the soaps can be decomposed by means of glacial acetic acid. The pigments are called *chromophanes*—the green one, chlorophane; the yellow one, xanthophane; [2] the red one, rhodophane. These pigments belong to the class of pigments called lipochromes, and their spectroscopic appearances (fig. 75, spectra 3, 4, and 5) should be compared with those of other lipochromes we have considered before (compare serum-lutein, p. 254; tetronerythrin, p. 325, and lipochrin, p. 459). Chlorophane shows two absorption bands; xanthophane and rhodophane each show one. The position of these bands shifts a little according to the solvent used, but, as in all other lipochromes, the bands are towards the blue end of the spectrum. There is always, in addition to the bands, a considerable absorption of the violet extremity of the spectrum.

Like other lipochromes the chromophanes, when evaporated to dryness, give the following colour reactions:—

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[1] Kühne and Ayres, *Journ. Physiol.* i. 109.

[2] Xanthophane is not identical with lipochrin obtained from the frog's hexagonal epithelium; the two pigments differ in solubilities and spectroscopic appearances.

1. Concentrated sulphuric acid; the fragments undergo a series of colour changes, being first a dirty green, then bluish green, and lastly violet. Later still this fades and only a brownish colour is left.

2. Concentrated nitric acid; a distinct bluish green colour is developed which lasts only a few seconds; then the flakes become colourless.

3. Iodine dissolved in solution of potassium iodide; the solution used is of this composition, iodine 0·25 gramme, potassium iodide 0·5 gramme, and distilled water 100 c.c. (Capranica).[1] This gives no colour reaction at all with the solid pigment. After the saponification of the pigment by the addition of strong caustic soda to the alcoholic solution, the iodine solution gives a bluish-violet colour, which, like the nitric acid colour, is evanescent.

Although Kühne speaks of these colours as the stable colours of the retina, he expressly points out that the word 'stable' is used only in a comparative sense; they are ultimately bleached by light; and this we have seen occurs in all other lipochromes, but much more slowly than in visual purple. A solution of chlorophane in a 5 per cent. solution of bile salts fades most rapidly; then one of xanthophane; while solutions of rhodophane are but little affected. In all cases, however, several days' exposure to sunlight is necessary for the bleaching process to become apparent. Indeed, some other lipochromes not connected with the eye at all, such as, for instance, that occurring in yolk of egg, bleach more quickly than the chromophanes.

Kühne was unable by exposing the retinæ of living birds to the sun for several hours to produce any change in the colours of the cone-globules. He, however, considers it possible that a slight change may be produced, so slight as to escape observation, but sufficient to act as a visual excitation. He is inclined, indeed, to class the chromophanes with visual purple, and certain colourless substances not yet separated, as visual substances or visual excitants; and, alluding to Hering's theory of the existence of three visual substances concerned in colour vision, he points out that the coloured oil-globules represent half the spectral colours, i.e. from the red to the yellowish-green, so that with their complementary colours they yield all the colours of the spectrum (Kühne).[2]

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[1] These first two colour reactions were established as general tests for lipochromes by Capranica, 'Physiologisch-chemische Untersuchungen über die farbigen Substanzen der Retina,' *Arch. f. Anat. und Physiol.* 1877, p. 283; and the iodine test by Schwalbe, 'Handbuch der gesammten Augenheilkunde,' *Gräfe u. Sämisch*, Bd. i. Th. i. Cap. iv. p. 414, Leipzig, 1874. The sulphuric acid and iodine reactions were first described for lutein by Piccolo and Lieben, 'Studii sul corpo luteo della vacca,' *Giornale di scienze naturali ed economiche*, anno ii. vol. ii. p. 258, Palermo, 1866. And the nitric acid reaction was first pointed out as being possessed by lutein by Thudichum, 'Ueber das Lutein &c.' *Centralbl. f. d. med. Wissenschaft*, Bd. vii. 1869, p. 1.

[2] *Journal of Physiology*, i. 189.

CHAPTER XXII

THE CONNECTIVE TISSUES

INTRODUCTORY

A LARGE number of tissues are grouped together under the heading Connective Tissue ; they may be classified as follows :—

1. Connective tissues proper :
    - Areolar tissue.
    - Fibrous tissue.
    - Elastic tissue.
    - Jelly-like connective tissue.
    - Retiform tissue.
    - Adipose tissue.
2. Cartilage.
3. Bone and dentine.

At first sight these tissues appear to form a heterogeneous group, but farther examination shows that the resemblances are sufficient to justify a grouping of them together. The similarities may be tabulated in the following way : —

1. Embryological : all are derived from the mesoblast.
2. Functional : all have a connecting or supporting function.
3. Histological : there are many points of structure in common. The histological elements are a ground substance or stroma containing cells and fibres ; the latter may, however, be absent, as in hyaline cartilage ; and the structure may be, as in bone and dentine, masked by calcareous deposit.
4. Chemical : all varieties of connective-tissue that contain white fibres yield gelatin, and the substance called chondrin yielded by cartilage is very similar.

The histological elements of connective tissue are four in number : –

1. Cells : connective tissue corpuscles of various kinds, cartilage cells, and cells of various kinds found in bone and dentine.
2. White fibres : immeasurably fine fibres which do not branch. which run in bundles ; these bundles have a wavy outline. The fibres

are not elastic; they are rendered transparent by acetic acid, and the substance of which they are composed (collagen) is converted into gelatin by the action of boiling water.

3. Elastic fibres: these are much thicker than the white fibres. They are angular in transverse section; they run singly, branching and anastomosing with one another; as their name implies, they are elastic; when massed together in large numbers they have a yellow colour, hence they are sometimes called yellow fibres. The substance of which they are composed is called elastin, which is a very insoluble material and but little affected by acids; hence, in a microscopic preparation, if the white fibres are rendered transparent by a little 1 per cent. acetic acid, the yellow or elastic fibres remain unaltered. The elastic fibres, in addition, may be distinguished by the readiness with which they are stained by magenta.

4. Ground substance, or intercellular substance. This is the material in which cells and fibres are imbedded; it has a jelly-like consistency, and its chief constituent is mucin. Like the cement substance of epithelium it is stained black by silver nitrate.

These different histological elements are differently distributed in the different connective tissues, and hence we are able to obtain a quantity of any one sufficient for chemical investigation from the tissue where that particular anatomical element preponderates in amount over the others; thus, elastin would be prepared from elastic tissue; collagen from fibrous tissue, and so on.

Keeping in view the enumeration of the histological elements found in the connective tissues, the following facts may be added concerning each individual tissue in our list:—

1. *Areolar tissue.*—This forms the subcutaneous tissue, the submucous and subserous tissue, the investing sheaths of organs, binding parts of organs to one another and different organs one to another. It is thus universally distributed. It contains all four histological elements, cells, and both kinds of fibres imbedded in ground substance.

2. *Fibrous tissue.*—This is the tissue that is found in tendons and ligaments, in the true skin, and in the denser fasciæ. It consists chiefly of bundles of white fibres arranged parallel one to another, giving to fibrous tissue in this way its great strength. Cells, ground substance, and a few elastic fibres are also present, but are relatively less important quantitatively.

3. *Elastic tissue.*—The ligamentum nuchæ of quadrupeds and the ligamenta subflava are composed of this tissue. Elastic structures are also found in the walls of the tracheæ and its branches, and in blood vessels. It consists chiefly of large elastic fibres; the other connective-tissue elements are relatively unimportant.

4. *Jelly-like connective tissue* is found in the vitreous humour of the eye, and in the Whartonian jelly of the umbilical cord. It consists almost entirely of

the ground substance of connective tissue, cells and fibres being either almost absent, as in the vitreous humour, or few and far between, as in the umbilical cord.

5. *Retiform and Lymphoid tissues.*—Lymphoid tissue is retiform tissue with lymph-cells in the meshes of the network; we have already considered this tissue in connection with the chemistry of lymph-cells and white blood-corpuscles (p. 258); the retiform tissue itself consists of a network of branching connective tissue-cells, many of which have lost their nuclei, and a few white fibres; any large development of the fibrous element and the ground substance is, however, wanting.

6. *Adipose tissue*, or fat, is, like areolar tissue from which it is developed, nearly universal in its distribution. It again is a tissue in which the cellular element is developed so as to preponderate over the fibrous; the cells are, however, not protoplasmic, but their protoplasm has become replaced or filled by fat; a flattened nucleus is, however, still discernible.

7. *Cartilage.*—Hyaline cartilage is found in the rib cartilages, the articular cartilages, the tracheal, laryngeal, and nasal cartilages, and also in the rods of cartilage which precede the long bones in the early stages of development. It consists of cells called cartilage cells imbedded in a clear matrix, which is much firmer than the ground substance of connective tissue, and which is composed of a substance called chondrigen; chondrigen is converted by the action of boiling water into chondrin, as collagen is converted into gelatin.

In some forms of cartilage, this matrix is more or less pervaded by white fibres or by yellow fibres; white and yellow fibro-cartilage are thus respectively produced; the former is found in the intervertebral discs and other interarticular cartilages, the latter in the epiglottis and the cartilage of the pinna of the ear. The histological and chemical characteristics of the white and yellow fibres in those situations resemble those of the fibres with the same names occurring in connective tissue proper.

8. *Bone.*—A bone consists of a fibrous membrane on the outside called the periosteum; then comes the osseous tissue proper, which consists of lamellæ wrapped concentrically around canals (Haversian canals), which contain the blood-vessels. Each lamella consists of a fibrous interior part (Sharpey's intercrossing fibres) imbedded in calcareous matter; the lamellæ are connected together by fibres passing through them at right angles to their surfaces; these are called Sharpey's perforating fibres; they are especially numerous in the neighbourhood of attached tendons, and appear to be calcified white fibres; some, however, which branch and stain readily with magenta are, perhaps, elastic in nature. Between the lamellæ, in spaces called lacunæ, the branches of which (canaliculi) anastomose, are the bone corpuscles. Other forms of cells are also found; those in connection with the periosteum, and chiefly instrumental in the formation of bone, are called osteoblasts; and others, similar to the large cells or myeloplaxes of marrow, are concerned in the eating away of bone, and are called osteoclasts. The marrow itself in the interior of the long bones is chiefly fatty tissue; in the spaces of cancellated bone it is designated red marrow; this contains, in addition, a number of cells which are coloured by hæmoglobin.[1] The function of this tissue we have already considered in connection with the

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[1] Lactic acid (Gorup-Besanez, *Phys. Chem.* p. 631) and hypoxanthin (Heymans, *Pflüger's Archiv*, vi. 184) have been described as occurring in small quantities in marrow.

development of red blood-corpuscles (p. 265) and in certain forms of leucocythæmia (p. 302). In birds the interior of many of the bones is filled with air.

Some bones are preceded by prefigurements in hyaline cartilage; this is the case with the long bones. The cartilage becomes first calcified; the primary bone or calcified cartilage so formed is eaten away almost entirely by osteoclasts and then replaced by true bone formed from the periosteum or perichondrium, as the investing membrane may be called at this stage. The osteoblasts appear first to deposit fibrous structures of a non-calcified nature; these are termed osteogenic fibres, and are apparently collagenous in nature; they form the intercrossing fibres of Sharpey. In the development of the so-called membrane bones (e.g. the flat bones of the skull, clavicle, &c.) there is no prefigurement of the adult bone in cartilage, but we have merely to deal with a subperiosteal deposition of osteogenic fibres, and this formation in the case of both varieties of bone is subsequently calcified by the agency of the osteoblasts which deposit calcareous granules around them till a complete investment is formed.

In some animals, like the elasmobranch fishes (rays, sharks, dog-fishes), calcified cartilage remains in the adult and is never replaced by subperiosteal bone. In invertebrate animals, bone is not found. Cartilage is occasionally found in invertebrates (Sepia, Limulus, Spirographis, &c.).

9. *Dentine.*—This substance forms the chief part of teeth; it is a calcified form of connective tissue; it is developed by the agency of certain cells called odontoblasts, and the deposition of calcareous matter is in process of development preceded by the formation of a non-calcified substratum which is termed odontogen.

Enamel is a calcified form of epithelium; it will, however, be convenient to consider it here with the other calcified structures of the body.

The scales of fishes may also be conveniently studied in this connection.

In all these hardened tissues the chief salt which is present is calcium phosphate.

In such a summary of the characters of the various forms of connective tissue as that just given, we see indicated the chief points which it will now be our object to consider more fully from a chemical point of view.

We shall have to deal first with the cells of connective tissue, then with the white fibres (collagen and gelatin), then with the elastic fibres (elastin), and lastly with the ground substance (mucin). In connection with adipose tissue we have the important subject fat to consider; in connection with cartilage, chondrigen, and chondrin; and in connection with bone and other calcified structures we shall have to take up both the organic and inorganic constituents.

Most of the organic constituents found in connective tissue belong to the important but somewhat heterogeneous group of the albuminoids, a class of substances closely related to the proteids. In studying each of these albuminoids, collagen, gelatin, mucin, elastin, chondrin, &c. it will be necessary to consider their mode of preparation, their chemical and physical characters, and their physiological meaning and importance.

THE CELLS OF CONNECTIVE TISSUE

The cells of connective tissue vary much in shape and histological appearances; some are branched and some not; some are vacuolated and others filled to a greater or less extent with fat-globules, this being especially the case in adipose tissue, or in areolar tissue, which is being converted into fatty tissue. The cells also differ functionally; they are most active in developing bone, and the different activities of osteoblasts, osteoclasts, and odontoblasts have already been alluded to. The knowledge of the chemical properties of the cells is, however, very limited; the way in which they are sparsely scattered through the tissue necessarily renders chemical investigation very difficult. The protoplasm of the cells consists chiefly of proteids, and their nuclei of nuclein, or of a mixture of nucleins (phosphorised nitrogenous substances).

The cells of the cornea are very contractile, and Kühne[1] surmised that they are closely related in composition to muscular substance. This was confirmed by Bruns,[2] who obtained myosin from the cornea, doubtless derived from its corpuscles.[3]

By some histologists the formation of connective tissue fibres is considered due to a deposition in the ground substance; this deposition may, however, be influenced by the cells in some way or other; others consider that the fibres are formed by the direct conversion of the cell protoplasm into fibrous material. The former view is, however, the one generally held.[4]

THE WHITE FIBRES OF CONNECTIVE TISSUE

The white fibres consist of a substance called collagen, and this, by boiling or by treatment with acids, is converted into gelatin.

Collagen

Collagen may be prepared in the following way: finely divided tendons are soaked in water to remove proteid substances, then in lime-water to remove the interfibrillar ground substance (mucin); they are then washed with water, dilute acetic acid, and finally water again. The residue is collagen. By the action of dilute acids or of boiling water collagen is converted into gelatin. Hofmeister[1] found that gelatin can be transformed into collagen by heating it to 130° C. By this treatment it loses water, and the reaction may be represented by the following formula:—

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[1] Kühne, *Unters. ü. das Protoplasma*, p. 123.

[2] Bruns, *Hoppe-Seyler's Med. Chem. Untersuch.* p. 260.

[3] The following points may here be added concerning the chemistry of the cornea. The epithelium is protoplasmic; the posterior homogeneous membrane is elastic in nature. The greater thickness of the cornea is composed of about sixty layers of alternating lamellæ of fibrous tissue (Bowman). This like other fibrous tissue is collagenous in nature, the interfibrillar and intercellular substance being composed of mucin. The anterior homogeneous lamella is similar in structure. The erroneous idea that the cornea contained chondrin was first fully pointed out by Morochowetz (*Verhandl. d. naturhist.-med. Vereins Heidelberg*, vol. i. part v.); 1000 parts of corneal tissue contain 242 of solids, of which 204 consist of collagen, 28 of other organic matters, 10 of ash (His). The sclerotic is also ordinary connective tissue. In birds, however, it contains bony plates.

[4] *See* Quain's *Anat.* 9th edit. ii. 70, 71.

$$\underset{\text{[gelatin]}}{C_{102}H_{151}N_{31}O_{29}} - H_2O = \underset{\text{[collagen]}}{C_{102}H_{149}N_{31}O_{28}}$$

in other words, collagen is the anhydride of gelatin. As will be seen by the above formula, gelatin, unlike proteids, contains no sulphur. Schützenberger, who gives the empirical formula $C_{76}H_{124}N_{24}O_{29}$ to gelatin, also regards the sulphur described by other investigators as being due to admixture with proteid impurities. The chief organic substance in bone (ossein) is identical with collagen.

Gelatin

Preparation.—Gelatin may be obtained from the white fibres of connective tissue either by treatment with dilute acids, by treatment with boiling water, or, best of all, by treatment with water in a Papin's digester at the temperature of 110–120° C. Bones, when similarly treated, also yield gelatin.

Hofmeister prepares pure gelatin from commercial gelatin by soaking the latter for several days in distilled water which is frequently changed. The salts pass out by osmosis. The gelatin is then dissolved in hot distilled water and filtered while hot (by the aid of a hot-water funnel) into 90 per cent. alcohol. As the hot solution falls into the alcohol it is precipitated; the alcohol is then evaporated off, and the process may then be repeated, and finally gelatin is obtained so pure that it only contains 0·6 per cent. of ash. This process may very conveniently be employed for the preparation of pure gelatin for cultivations of bacteria.

Properties.—Gelatin is insoluble in cold water, but soluble in hot water; the hot aqueous solution sets into a jelly when cold. This property of jellying or *gelatinising* is possessed by solutions containing 1 per cent. of gelatin or upwards. With every successive solution, however, the power of gelatinising is lessened; it is entirely destroyed by twenty-four hours' boiling with water; it is more quickly lost at higher temperatures and instantly lost at the temperature of 140° C. In order to raise an aqueous solution of gelatin to these temperatures, it is necessary to employ either a digester or sealed tubes. Gelatin is insoluble in cold glycerine and soluble in hot glycerine; such a hot solution gelatinises on cooling (glycerine jelly) like an aqueous solution.

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[1] Hofmeister, *Zeit. physiol. Chem.* ii. 315.

Gelatin is insoluble in alcohol, ether, and chloroform.

Gelatin is precipitated by saturating its solutions with neutral salts, like magnesium sulphate or ammonium sulphate (O. Nasse).[1] This is also true for gelatin which has been altered by the action of hot water so as to be no longer or only partially gelatinisable.

Aqueous solutions of gelatin are powerfully lævorotatory; the amount of rotation varies, however, like the power of gelatinisation, according to its treatment with hot water and also according to the temperature. At 30° C. $(\alpha)_D = -130°$ (Hoppe-Seyler).

Solutions of absolutely pure gelatin transmit a perfectly continuous spectrum as far as wave-length 2024; impure solutions and films of gelatin will not transmit rays nearly so far into the violet, and the sensitiveness of photographic plates to the most refrangible rays lies entirely with the character of the gelatin (Hartley [2]).

Gelatin is not precipitated by acetic acid. Hence it can be readily distinguished from mucin or chondrin.

It is not precipitated by acetic acid and ferrocyanide of potassium, by lead acetate, nor by most of the heavy metallic salts that precipitate proteids. Hence it can be readily distinguished from proteids. It gives a violet colour with copper sulphate and caustic potash like proteids, but it gives only a faint xanthoproteic reaction (Salkowski[3]).

Gelatin is precipitated by tannic acid and also by mercuric chloride. The compound with tannic acid is an interesting one, as it is the formation of this substance during the process of tanning that converts hides into leather.[4]

Derivatives of gelatin.—The prolonged action (twenty-four hours) of boiling water, or the shorter action of water heated above the boiling point, destroys the gelatinising power of gelatin. It undergoes a process of hydrolysis. In peptic digestion a similar change occurs. In pancreatic digestion the same products are formed, but they undergo, as proteid peptones do, a partial decomposition into amido-acids and other simpler substances. The gelatin peptones, as they are termed, formed by these different processes are really more akin to the proteoses than to true peptone. They are precipitable by saturation with magnesium sulphate or ammonium sulphate. Like the gelatin from which they are derived they give the xanthoproteic reaction very faintly.

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[1] O. Nasse, *Pflüger's Archiv*, xli. 504.

[2] Hartley, *Trans. Chem. Soc.* 1887, p. 59.

[3] E. Salkowski, *Zeit. physiol. Chem.* xii. 215; *Ber. klin. Wochensch.* 1888, No. 2.

[4] For particulars of the chemical properties of the compound of tannic acid and gelatin, see Böttinger, *Annalen f. Chem. u. Pharm.* ccxliv. 227.

Hofmeister distinguishes two of these peptone-like substances; one he terms *semi-glutin* is insoluble in 70–80 per cent. alcohol, and is precipitated by platinum tetrachloride; the other, called *hemi-collin*, is soluble in 70–80 per cent. alcohol, and is not precipitable by platinum tetrachloride.

The following equation exhibits the relationship of these substances to collagen:—

$$\underset{\text{[collagen]}}{C_{102}H_{149}N_{31}O_{38}} + 3H_2O = \underset{\text{[semi-glutin]}}{C_{55}H_{85}N_{17}O_{22}} + \underset{\text{[hemi-collin]}}{C_{47}H_{70}N_{14}O_{19}}$$

Chemical constitution of gelatin.—The formulæ that have been given to gelatin by different observers are purely empirical; as in the case of proteids, though there are several theories as to the constitution of the molecule, we are practically entirely ignorant on this point.

Strong agents, like sulphuric acid or putrefaction, decompose the substance, forming glycocine, leucine, various fatty acids, carbon dioxide, and ammonia. Schützenberger[1] heated gelatin in sealed tubes with baryta-water to 200° C. The products he obtained were ammonia, carbonic acid, and oxalic acid, these compounds being in the ratio of the products of decomposition of urea. The other products were amido-acids of the acetic series, the most abundant in the order of their importance being glycocine (amido-acetic acid), alanine (amido-propionic acid), amido-butyric acid, and leucine (amido-caproic acid). The general conclusion is drawn that gelatin, like a proteid, is a compound of urea with certain amido-acids.

It will be noticed in all these decompositions no substances like tyrosine containing aromatic radicles are obtained as they are from proteids.

THE ELASTIC FIBRES OF CONNECTIVE TISSUE

The elastic or yellow fibres, the microscopical characters of which have been already described, consist chemically of the very insoluble albuminoid which is called elastin. During development, elastic granules are deposited either in rows, which subsequently become fused together end to end to form fibres, or they may be deposited in patches, and by their fusion form an elastic membrane, as in the elastic (fenestrated) membrane of arteries. The elastin granules make their appearance first in the neighbourhood of the cells; this renders it probable that the deposition of the granules is influenced by the cells, but it does not prove that they are formed by a direct conversion of the cell-protoplasm.

Elastin

Preparation.—As usually described elastin is an albuminoid substance which, like gelatin, is free from sulphur, but which offers great resistance to the action of reagents. The method of preparing this material from tissues which contain a large quantity of it (such as the ligamentum nuchæ of the ox, horse, or giraffe), consists in treating the finely divided ligaments successively with reagents in which it is insoluble, and in which adherent collagenous and proteid substances readily dissolve. The ligaments are treated first with boiling water, then with 1 per cent. potassium hydroxide solution, then in 10 per cent. acetic acid, then in 5 per cent. hydrochloric acid, and lastly with alcohol and ether. This method of purification takes several days, large excess of each of the reagents mentioned must be employed, and each fluid must be changed two or three times.

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[1] *Comptes rendus*, cii. 1296. For some work on the decomposition of gelatin *see* also Buchner and Curtius, *Berichte d. chem. Gesellsch. Berlin*, xix. 850.

Composition.—By this means a substance free from sulphur is obtained. Chittenden and Hart[1] in some of their preparations of elastin omitted the extraction with potash, and in these a small percentage of sulphur (0·3 per cent) was found; it is, however, doubtful whether this is really in the elastin molecule or in proteid matter which is present as an impurity.

The following table shows the results in percentages of elementary analysis by different observers:—

	Müller[2]	Tilanus[3]	Horbaczewski[4]	Chittenden and Hart
Carbon . . .	55·09–55·72	54·90–55·65	54·32	54·24
Hydrogen . . .	7·11– 7·67	7·25– 7·41	6·99	7·27
Nitrogen . . .	15·71–16·52	17·52–17·74	16·75	16·70
Oxygen . . .	20·7 –21·15	19·50–20·33	21·94	21·69

The higher content of carbon in the preparations of Müller and Tilanus is doubtless due to the presence of more or less fat, not completely extracted with ether.

Properties.—Elastin is not soluble in any liquid that does not decompose it. It is soluble in hot concentrated caustic potash, in cold concentrated sulphuric acid, and also in cold concentrated nitric acid.

When boiled with sulphuric acid elastin yields leucine, but no tyrosine.

When digested with pepsin or trypsin, elastin is gradually but slowly dissolved. The older writers looked upon it as being almost insoluble in the digestive juices. Kühne and Ewald[5] appear to be the first who obtained a solution of elastin by digestive agents, and these observers noticed that pepsin was more active than trypsin. More recently Etzinger,[1] Horbaczewski,[2] and Morochowetz[3] have shown that finely divided ligamentum nuchæ, or powdered purified elastin, are fairly soluble in artificial digestive juices, and Horbaczewski was able to verify this with natural gastric juice obtained from a man with a gastric fistula.

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[1] Chittenden and Hart, *Zeit. Biol.* xxv. 368; *Studies from the Lab. of Physiol. Chem. Yale University*, iii. 19.

[2] Müller, *Zeitsch. f. rat. Med.* 3rd series, vol. x. part ii.

[3] Tilanus, *Gorup-Besanez' Physiol. Chem.* 3te Aufl. p. 148.

[4] Horbaczewski, *Zeit. physiol. Chem.* vi. 330.

[5] Ewald and Kühne, *Die Verdauung als histol. Methode.*

Horbaczewski named the two products of digestion hemi-elastin and elastin-peptone. Chittenden and Hart,[4] who repeated these experiments, using the methods adopted by Kühne and Chittenden in the examination of the digestion products of proteids, found that both these substances are analogous rather to the proteoses, the intermediate stages in the formation of peptone, than to true peptone; they are both, for instance, precipitable from their solutions by saturation with ammonium sulphate, while true peptone is not. They have named the two products of digestion of elastin, proto-elastose (this corresponds to Horbaczewski's hemi-elastin) and deutero-elastose (this corresponds to Horbaczewski's elastin-peptone); the former is precipitable by saturation with sodium chloride, the latter is not. Both are precipitable by saturation with ammonium sulphate, and both give the xanthoproteic and biuret tests. The names just mentioned are analogous to the names of the albumoses, the first cleavage products in the digestion of albumin. These same substances can be obtained from elastin by the action of trypsin, or by the prolonged action of acidulated water at 100° C.

THE GROUND SUBSTANCE OF CONNECTIVE TISSUE

The ground substance of connective tissue, like the cement-substance of epithelium, has the power of forming a compound with silver salts, which becomes reduced in the light, and consequently brown or black from the deposit of metallic silver in it. This property is of great value to the histologist, as a means of demonstrating the spaces in the ground substance in which the cells lie. These spaces, which are connected to one another, form a branching network of irregular canals (*Saft Kanälchen*) in which lymph circulates.

The chief constituent of the ground substance is mucin. This is readily soluble in lime-water or other weak alkalis, and so the various tissue elements of fibrous tissue and other forms of connective tissue fall apart when they are treated for about twenty-four hours with lime-water, owing to the solution of mucin. The other organic constituent of the ground substance is a proteid; this occurs in small quantities; it belongs to the class of proteids (which are insoluble in distilled water, soluble in dilute saline solutions, and insoluble in saturated saline solutions) called globulins. It is coagulated by heating its solution to 75° C.

Mucin is obtained in greater abundance from embryonic connective tissues than from those of the adult; in the fully formed connective tissues the ground substance is very largely replaced by fibrous (collagenous) material. In the vitreous humour[1] and Whartonian jelly of the umbilical cord, in which the fibrous and cellular elements of connective tissues are reduced to a minimum, mucin can be obtained in abundance.

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[1] Etzinger, *Zeit. Biol.* x. 84.

[2] *Loc. cit.*

[3] Morochowetz, *Maly's Jahrsbericht*, 1886, p. 271.

[4] *Loc. cit.*

Mucin

Mucin is a substance which has a slimy consistency, and of which there are several varieties. It is found, not only in the ground substance of connective tissue and the cementing substance between epithelial cells, but, as we have already seen, in many epithelial structures (see Mucus, p. 444). We shall also have to consider it in a few secretions, such as the saliva. It must be carefully distinguished from certain nucleo-albumins which have similar physical characters, such as, for instance, the so-called mucin of the bile. Mucin, again, is an ingredient of the tissues of certain invertebrates, and the mucin obtained from the snail has been studied by Eichwald and Hammarsten.

Elementary analysis has shown that mucin from different sources differs in composition very much. Many of the minor reactions of the substance also vary, and it is now pretty generally granted that different mucins differ from one another in the nature of the proteid which is combined with a carbohydrate radicle to form the mucin molecule. The name given by Landwehr[2] to the carbohydrate which may be obtained from the various forms of mucin is animal gum.

Hammarsten[3] gives the following three properties as characteristic of a mucin :—

1. Its viscidity and stickiness.
2. Its solubility in dilute alkalis; it is precipitable from such solutions by acetic acid, being insoluble in excess of that reagent.
3. When heated with dilute sulphuric acid, it yields a reducing sugar.

Connective-Tissue Mucin

Preparation.—The different methods that have been adopted for the preparation of mucin from connective tissue are all essentially the same, though they differ in detail (Rollett,[4] Lœbisch[5]). The tissue is finely minced, washed with water, and then extracted for twenty-four to forty-eight hours with a very large excess of lime-water, or baryta-water diluted to five times its bulk with distilled water. The extract is then precipitated with excess of acetic acid, the precipitate is allowed to stand a few hours; in this time it collects into large flocculi or stringy masses if a large quantity of mucin is present, as when one is dealing with the vitreous humour. The substance is collected and may be purified by redissolving it in lime-water, filtering, and reprecipitating it from the filtrate by acetic acid.

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[1] Gorup-Besanez (*Physiol. Chem.*) gives the following analysis of the vitreous humour by Lohmeyer: water in 1000 parts, 986·4; membranes 0·21; proteids and mucin 1·36; fats 0·016; extractives (urea &c.) 3·2; sodium chloride 7·76; other mineral matters 1·05.

[2] Landwehr, *Zeit. physiol. Chem.* vols. vi. vii. viii. ix.

[3] *Chem. Centralbl.* 1884, p. 814.

[4] Rollett, *Stricker's Handbuch*, i. 72.

[5] Lœbisch, *Zeit. physiol. Chem.* x. 40.

Some recommend that the tissue should be first placed in alcohol for a week or two to coagulate the proteids that are present. This, however, is quite unnecessary; for if any proteid is dissolved by the lime-water it is precipitated, as alkali-albumin always is, by the acid, but is readily soluble in excess. The spirit has the disadvantage of rendering the pieces of tissue hard, and so they cannot be permeated easily by the lime-water; ultimately, also, it renders mucin insoluble.

Instead of using lime-water or baryta-water, other weak alkalis, like a 1 per cent. solution of sodium carbonate, may be employed; or even distilled water, no doubt in virtue of the alkaline salts in the tissue, will extract a considerable quantity of mucin.

Estimation of mucin.—The amount of tissue taken is weighed in the first instance; it is extracted with lime-water repeatedly till no more mucin goes into solution; and the mucin precipitated from this by acetic acid is collected on a weighed filter and washed with 2 per cent. acetic acid, distilled water, alcohol, and ether; it is finally dried, weighed, and incinerated, the amount of ash being deducted. The percentage of mucin can be thus calculated. In view of the alleged increase of mucin in a disease known as myxœdema, it is important to have certain data concerning the amount present in normal tissues. Although the above method cannot claim to be absolutely accurate, it gives very good comparative results. On p. 478 is the result of a number of analyses made by Dr Stevenson and myself.[1] In all the cases there enumerated the percentages refer to the organ as weighed in the fresh condition.

The following numbers represent the averages obtained in normal tissues. The details are given in the tables on the next page.

	Percentage of mucin
Skin (children)	0·766
Skin (adults)	0·385
Connective tissues	0·521
Parotid Heart tendons	traces

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[1] *Clin. Soc. Transactions*, vol. xxi. supplement, report of Myxœdema Committee.

Skin

Sex of patient	Age of patient	Cause of death	Part from which the skin was taken	Percentage of mucin present
M.	Stillborn	—	Leg	0·96
M.	7 weeks	Pyæmia	Abdomen	1·02
F.	2 years	Syphilis	"	0·74
M.	6 "	Gangrene of lung	"	0·72
F.	9 "	Nephritis	"	0·39
M.	20 "	Tuberculosis	"	0·42
F.	40 "	Carcinoma	"	0·29
M.	53 "	—	"	0·11
M.	56 "	Aortic disease	"	0·64
M.	63 "	—	"	0·264
F.	67 "	Pneumonia	"	0·59

Other Tissues

Case	Tissue	Percentage of Mucin	Analyst
Bright's disease	Connective tissue from thigh (?)	0·5	Stevenson ('Clin. Soc. Trans.' xv. 94)
Case of accident	*a.* Connective tissue from thigh	0·5	Ditto
	b. Tendo Achillis	0·77	
	c. Tendons of breast *d.* Pericardial fat	Traces	
—	Heart tendons	Doubtful	Dr. Stevenson (*Ibid.* xxi. suppl.)
Female (Bright's disease)	Tendo Achillis	0·539	Halliburton
Male (septicæmia)	Tendo Achillis	0·298	
—	Parotid	Trace	

The high percentage of mucin in the incompletely developed connective tissue of young children is due to the greater quantity of ground substance present there.

In cases in which fluids are to be analysed for mucin, the precipitate produced by the addition of acetic acid may be collected and weighed, or alcohol may be added to the fluid; this precipitates both mucin and proteids; the former may then be dissolved out from this precipitate by means of baryta-water or lime-water, and precipitated therefrom by acetic acid.

Lœbisch states that mucin has an acid reaction, and that the amount present may be measured by the decrease of alkalinity of an alkaline solution employed to dissolve it.

Properties and reactions of connective-tissue mucin.—Mucin is a slimy, glutinous substance insoluble in water and in alcohol. It is soluble in weak alkaline solutions, such as lime-water, from which it is easily precipitable by acetic acid, and is not soluble in excess of that acid. It may also be precipitated by means of the mineral acids, but is soluble in excess of those reagents. It is insoluble in solutions of hydrochloric acid containing less than 1 per cent. of the acid. In 2 per cent. hydrochloric acid it dissolves slowly, but more quickly on the application of heat. In 5 per cent. hydrochloric acid it dissolves readily. It, however, loses its characteristic properties when treated with acids of this strength, being split into its proteid and carbohydrate constituents, the former being converted into acid-albumin.

Mucin is precipitated from its solutions completely by saturating them with sodium chloride, magnesium sulphate, or ammonium sulphate.

Mucin which has been precipitated by acids is almost insoluble in solutions of common salt (5–10 per cent.), but mucin as it exists in the vitreous humour is more soluble in such solutions, but still not freely soluble.

The various mucins differ from one another as to whether or not they are precipitated by various metallic salts; connective-tissue mucin is precipitated by lead acetate, by ferric chloride, and by copper sulphate. It is not precipitated by potassium ferrocyanide and acetic acid; it is not precipitated by tannic acid.

With copper sulphate and caustic potash it forms a violet solution, which is not reduced on boiling. With Millon's reagent it behaves like a proteid. It also gives the xanthoproteic reaction (orange colour with nitric acid and ammonia).

The prolonged action (six to eight weeks) of absolute alcohol renders mucin insoluble in dilute alkalis. It appears to be converted into a substance like coagulated proteid.

Composition of connective-tissue mucin.—From its percentage composition (C, 48·3; H, 6·44; N, 11·75; S, 0·81; O, 32·7) Lœbisch has calculated the following empirical formula: $C_{160}H_{256}N_{32}SO_{80}$. It thus contains the same elements as a proteid, and there is no doubt that mucin is an albuminoid which is very closely allied to the proteids, and that it is the product of the differentiation of the protoplasm of animal cells. Landwehr indeed considers it as only an intimate mixture of a proteid and a carbohydrate; but it is more generally regarded as a chemical unit, a compound of these two substances. The action of dilute mineral acid is first to split up the mucin into proteid and carbohydrate. The proteid is converted into acid-albumin; the carbohydrate (Landwehr's animal gum) has the empirical formula $C_6H_{10}O_5$. It does not reduce copper salts; by the further action of the mineral acid, the gum is converted into a reducing sugar or gummose, which has the formula $C_6H_{12}O_6$.

Decomposition products of mucin.—1. *The proteid constituent of mucin.*—This appears to be of the nature of a globulin; it is precipitated by salts like a globulin, and is convertible, like globulins, with extreme ease into acid-albumin. Mucin in suspension in water becomes very insoluble in weak alkalis after its temperature has been raised to 70° C. This is presumably because the heat-coagulation temperature of the proteid contained in the mucin has been reached.

2. *The carbohydrate constituent of mucin.*—*Animal gum.*[1]—This substance may be obtained from mucin in the following way: Mucin is dissolved in weak hydrochloric acid by the aid of heat; on neutralising with soda a white flocculent precipitate of proteid is obtained, which is increased in amount by the addition of sodium sulphate crystals and boiling. This is filtered off, and the filtrate contains animal gum; this may be precipitated by means of copper sulphate, and the copper subsequently separated from it Landwehr has obtained this carbohydrate from the mucin of tendon, of the saliva, of synovia, from colloid cysts, from metalbumin and paralbumin, from chondrin, and in small quantities from all parts that contain connective tissue, such as the brain, kidney, spleen, &c.; he has also separated it in small quantities from the urine.[2]

Animal gum forms an opalescent solution with water; it gives a sticky precipitate with copper sulphate, and also with ferric chloride. It does not reduce alkaline solutions of copper salts. It gives no colour with iodine. It is precipitated by alcohol. Like vegetable gum, it yields oxalic acid after treatment with nitric acid, and lævulic acid after treatment with hydrochloric acid. Boiling a solution with 1 per cent. sulphuric acid gives it the power of reducing alkaline solutions of copper and bismuth salts: this is due to the conversion of the gum ($C_6H_{10}O_5$) into gummose ($C_6H_{12}O_6$), a reducing substance, which will not, however, undergo the alcoholic fermentation. This conversion is, however, slow and incomplete.

The physiological and pathological importance of animal gum seems to be much exaggerated by its discoverer: the following are, however, the chief points advanced by Landwehr in this relation: Animal gum is present in the fœtal tissues more abundantly than in the adult; in some animals, as in the frog, the gum is derived from the mucinoid envelope of the egg,[3] in mammals from the mother; periods of activity of the generative organs are thus associated with an increased formation of animal gum; and pathological conditions of the female generative organs are often associated with excess of mucin and other compounds that contain gum, such as ovarian cysts and myxœdema.[4] The part that animal gum is supposed to play in chlorosis has been already dealt with (p. 300). Among other functions attributed to animal gum are: a part in the production of hydrochloric acid in the stomach; the aiding of the emulsification and absorption of fats in the intestine; it is also regarded as the mother substance of milk sugar. These statements await verification before they can be received as more than interesting theories.

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[1] Landwehr, *Pflüger's Archiv*, xxxix. 193; xl. 21.

[2] Landwehr, *Centralbl. med. Wissensch.* 1885, p. 369. This observation has been confirmed by Wedenski, *Zeit. physiol. Chem.* xii. 122.

[3] Wolfenden (*Journal of Physiology*, v. 91) shows that the envelope of the frog's egg consists of mucin.

[4] No constant relation has been shown, however, to exist between diseases of the generative organs and myxœdema (*see* further, next chapter).

Further decomposition products of mucin.—Mucin yields leucine and tyrosine after prolonged boiling with strong sulphuric acid. Obolensky[1] obtained pyrocatechin ($C_6H_6O_2$) by boiling submaxillary mucin with caustic soda for fifteen to twenty minutes; but I have not succeeded in obtaining this substance from connective-tissue mucin in this way. Putrefaction produces the same decomposition products as it does from proteids.

Mucin is not digested by artificial gastric juice; if mucin, however, be dissolved in 2 per cent. hydrochloric acid, and the solution diluted till the percentage of HCl is 0·2, and then pepsin be added, albumoses and peptones will be formed at a suitable temperature (40° C.); but, as has been explained already, a solution of mucin made in this way is not really a solution of mucin at all, but a solution of the decomposition products of mucin, one of which is acid-albumin; and this it is which is converted into peptone by the action of pepsin. Pancreatic juice, in virtue of its alkalinity, readily dissolves connective-tissue mucin, and the results of artificial pancreatic digestion are albumoses, peptones, leucine, tyrosine, &c. from the proteid part of the molecule, and a reducing sugar from the animal gum.

CARTILAGE

In hyaline cartilage the matrix is free from fibres. In it are embedded numerous cells, most commonly in groups of two or more; the cells are rounded except in the neighbourhood of adjoining fibrous tissue, where they are branched (transitional cartilage).

The matrix is much firmer than the ground substance of connective tissue proper, but, like it, is stained brown by nitrate of silver and subsequent exposure to light. It yields, on boiling, a substance called chondrin.

The cells lie in cavities in the matrix, which they apparently entirely fill in the natural condition. Each cavity is bounded and enclosed by a transparent capsule, which coheres intimately with the surrounding matrix, and it is only in young cartilage that it can be clearly distinguished from the matrix without the use of reagents. The cells in cartilage may be sometimes seen in a state of division, as indicated by the karyokinetic figures in their nuclei; the capsule divides with the cell, so that in a group of cells formed by subdivision a capsule may be traced surrounding the whole group, and secondary capsules enveloping each cell. It is doubtful how the capsule is produced, whether excreted by the cell which it subsequently encloses (Kölliker) or formed by a conversion of the superficial layer of the protoplasm of the cell-body (Max Schultze). There is at first no matrix but what is made up of the simple capsules.[1]

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[1] *Pflüger's Archiv*, iv. 336.

In fibro-cartilage the matrix, at first hyaline, is subsequently pervaded by elastic fibres (yellow or elastic fibro-cartilage), or by white fibres (white fibro-cartilage). The fibres here have the same properties and characteristics as those which occur in connective tissue proper.

Cartilage cells are rounded, oval, or bluntly angular masses of protoplasm embedded in which are fine curvilinear interlacing filaments and minute granules; by the use of osmic acid, fat-globules of varying size may often be demonstrated to exist; they are stained black by the osmic acid. Occasionally, and especially in young cartilage cells, glycogen appears to be present, for the cells are coloured brown with iodine (Neumann[2]).

The following analyses by Hoppe-Seyler[3] exhibit the relative proportions of water, and of organic and inorganic matters in human hyaline cartilage:—

	Costal cartilage	Articular cartilage
Water in 1000 parts	676·7	735·9
Solids	323·3	264·1
Organic solids	301·3	248·7
Inorganic solids	22·0	15·4

Inorganic Solids of Costal Cartilage

Potassium sulphate	in 100 parts of ash	26·66
Sodium sulphate	„ „	44·81
Sodium chloride	„ „	6·11
Sodium phosphate	„ „	8·42
Calcium phosphate	„ „	7·88
Magnesium phosphate	„ „	4·55

The organic solids consist of those in the cells, which are mostly of proteid nature, and the organic basis of the matrix, which yields the albuminoid substance chondrin, and forms the greater part of the organic material of cartilage.

Chondrin

Preparation.—Chondrin may be obtained from hyaline cartilage by finely dividing the latter, and heating it with water in a digester or sealed glass tube to the temperature of 120° C. The solution so obtained is filtered while hot to separate insoluble materials from it. A large excess of alcohol is added to the solution ; this precipitates the chondrin ; the precipitate is collected, well washed with alcohol and with ether, and may be still further purified by redissolving it in hot water, and reprecipitating it by means of alcohol. The precipitate so obtained is then dried ; it is hard, transparent and devoid of taste. The name chondrigen has been given to the mother substance of chondrin which exists in the cartilage.

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[1] For further particulars concerning the structure and development of cartilage see Quain's *Anat.* 9th edit. ii. 84.

[2] *Arch. f. mikr. Anat.* vol. xiv. 1877.

[3] Quoted by Gamgee, *Physiol. Chem.* p. 268.

Reactions.—The chief reactions of chondrin may be given in the following tabular form, and with them are placed also the corresponding reactions of mucin and of gelatin :—

	Chondrin	Gelatin	Mucin
Solubilities	Insoluble in cold water, alcohol, or ether	Insoluble in cold water, alcohol, or ether	Insoluble in cold water, alcohol, or ether
	Soluble in hot water ; such solutions set into a jelly when cold	Soluble in hot water ; such solutions set into a jelly when cold	Insoluble in hot water
Acetic Acid	Gives a precipitate insoluble in excess	Gives no precipitate	Gives a precipitate insoluble in excess.
Mineral Acids	Give a precipitate soluble in excess	Give no precipitate	Give a precipitate soluble in excess
Tannic Acid	Gives a precipitate	Gives a precipitate	Gives no precipitate
Mercuric Chloride	Gives a precipitate	Gives a precipitate	Gives no precipitate
Lead Acetate	Gives a precipitate	Gives no precipitate	Gives a precipitate
Alum	Gives a precipitate	Gives no precipitate	Gives a precipitate
When decomposed by boiling with dilute mineral acids	A reducing sugar is formed	No reducing sugar is formed	A reducing sugar is formed

This table shows that chondrin possesses the reactions of gelatin, and also those of mucin. As in the case of gelatin, the power of gelatinisation is lost after prolonged boiling.

Composition of chondrin.—The following table [1] represents the results in percentages of the elementary analyses that have been made of chondrin :—

Elements	Mulder	Fischer and Bödeker	Schützenberger and Bourgeois	v. Mering
Carbon	49·3	50·0	50·16	47·74
Hydrogen . . .	6·6	6·6	6·58	6·76
Nitrogen	14·4	14·4	14·18	13·87
Sulphur	0·4	0·4	0	0·6
Oxygen	29·3	28·6	29·08	31·04

It will be observed that great discrepancies exist between these various results, and point to a conclusion which we shall see to be fully justified, that chondrin is not a chemical unit but a mixture.

It was Morochowetz [2] who first arrived at the conclusion that chondrin is a mixture, and a mixture, as its reactions indicate, of gelatin and mucin. After treating cartilage with liquids like lime-water which dissolve mucin, the residue is on boiling readily converted into perfectly normal gelatin.

The reducing sugar obtainable from chondrin was at one time called chondri-glucose.[3] Landwehr has, however, shown that it is really a sugar obtained from animal gum, the carbohydrate constituent of the mucin present in the cartilage.

This discovery of Morochowetz, that chondrin is a mixture of gelatin and mucin, and that in consequence chondrigen is a mixture of collagen and mucin, is a point of some importance, as it shows that cartilage is no exception among connective tissues, as it has been supposed to be. It really has a collagenous basis like other connective tissues, but this is masked to some extent by the admixture with mucin.

Landwehr, Krukenberg, and C. T. Mörner [4] have since confirmed the general accuracy of Morochowetz' work. Mörner's observations, made under the superintendence of Prof. Hammarsten, are chiefly of a micro-chemical nature, and being of great interest may be quoted here. The cartilage investigated was that of the trachea; the existence of two substances in the matrix can be demonstrated by the use of certain staining reagents. Methyl violet, indigo red, and a mixture of ferric chloride and potassium ferrocyanide stain a trabecular network which pervades the matrix, and is continuous with the perichondrium. This is composed of collagen, and from it gelatin is obtainable. In late life a very insoluble proteid is present also. The other substance occurs in spherical masses (chondrin balls) which surround the cells, and correspond in position to the cell-capsules.

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[1] Quoted by Gamgee, *Physiol. Chem.* p. 270.

[2] *Verhandl. d. naturhist. med. Vereins zu Heidelberg*, vol. i. part v.

[3] Fischer and Bödeker, *Annalen der Chem. u. Pharm.* cxvii. 111.

[4] *Zeit. physiol. Chem.* xii. 396; *Skandinav. Archiv f. Physiol.* i. 210.

This is not stained at all by the reagents previously mentioned, but is stained by indigo blue, aniline red, and tropæolin, which do not affect the collagenous network. By the use of two staining reagents, one of each group, applied to microscopic sections, double staining is obtained; for instance, with methyl violet and tropæolin, the network is stained yellow, the chondrin balls blue; with indigo blue and aniline red the network is stained blue, the chondrin balls red. It is the chondrin balls which consist of the second substance of other authors, the mucin of Morochowetz and v. Mering, the hyalogen of Krukenberg. There are, however, some slight differences between the chondrin balls and ordinary connective-tissue mucin; they consist of two substances: (1) a mucin (*chondromucoid*) which yields on decomposition proteid matter and chondroitic acid; and (2) free *chondroitic acid* (*see* below).

Further decomposition products of chondrin.—On being heated with strong sulphuric acid, chondrin yields similar decomposition products to those obtained from gelatin and mucin. Hoppe-Seyler,[1] however, states that no glycocine is formed. Schützenberger and Bourgeois,[2] who employed baryta-water in sealed tubes as in their researches on proteids and on gelatin, obtained results similar to those derived from those substances.

A substance of uncertain nature, called chondroitic acid, was obtained by Bödeker[3] as a result of treating cartilage with certain reagents. This substance has also been examined by Krukenberg,[4] who ascribes to it the formula $C_{28}H_{31}SN_2O_{30}$, and believes it is one of a class of substances he terms hyalins (*see* further next page). Mörner found that this substance, which is remarkable for its low percentage of nitrogen, occurs free in the chondrin balls, and is also a decomposition product of chondro-mucoid. On being boiled with dilute sulphuric acid it yields a reducing sugar. Mörner was, however, not able to verify the existence of Krukenberg's hypothetical precursor of this substance or hyalogen. A fact of great interest made out by Mörner is that the sulphur in the molecule is all combined in the form of ethereal sulphate.

Cartilage in Invertebrate Animals

The cartilage occurring in certain invertebrate animals is very similar histologically to that in the vertebrate kingdom. It is also very similar chemically. Hoppe-Seyler was the first to obtain gelatin from it.[5] I[6] have myself made a chemical examination of the head cartilage of Sepia, and the entosternite of Limulus. The basis in both structures is chondrin; that is to say, a substance giving the reactions both of gelatin and mucin; there is, however, in addition, a certain proportion of chitin, in the case of Limulus 1·01, and in that of Sepia 1·22 per cent. These results are especially interesting, as showing that chitin is not a substance which is exclusively epiblastic in origin, but here, at least, we have it occurring in mesoblastic structures.[7]

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[1] *Journ. f. prakt. Chem.* lvi. 129.

[2] *Comptes rendus*, lxxxii. 262.

[3] *Annal. der Chem.* (1861), vol. cxvii. p. 111.

[4] Krukenberg, *Zeit. Biol.* xx. 307.

[5] Hoppe-Seyler, *Med. Chem. Untersuch.* p. 580; *Pflüger's Archiv*, xiv. 395; Krukenberg (*Zeit. Biol.* vol. xx. Heft 3) also worked at this subject.

[6] *Quart. Journ. Mic. Science*, xxv. 173; *Proc. Roy. Soc.* 1885, No. 235.

[7] I have also shown that chitin occurs in the liver of Limulus, though whether in the connective tissue or in the liver cells I am unable to say. Krukenberg (*Ber. d. chem. Gesellsch. Berlin*, xviii. 989) has since found chitin in the pen of Sepia.

Hyalins and Hyalogens

The term hyalin was originally applied to the principal constituent of the walls of hydatid cysts. The cysts are boiled with water, alcohol, and ether; the residue is then heated with water in a sealed tube to 150° C. The substance hyalin passes into solution. It is precipitable from this solution by alcohol and by lead acetate; on treatment with dilute sulphuric acid it yields a reducing sugar like mucin. Its percentage composition is as follows (Lücke[1]):—

	From young cysts	From old cysts
Carbon	44·1	45·3
Hydrogen	6·7	6·3
Nitrogen	4·5	5·2
Oxygen	44·7	43·0

In addition to the hyalin obtained in this way, Krukenberg[2] has extended the name to allied substances obtainable from other animal structures. These substances exist in the natural state as insoluble materials, and these he terms hyalogens: by the action of alkalis or of superheated water these are rendered soluble; and to the soluble substance so produced the generic term hyalin is applied. The names given to the individual substances are as follows:—

Hyalin	Hyalogen	Remarks
Neossidin	Neossin	The chief component of the edible birdsnest.[3]
Chondrosidin	Chondrosin	Obtained from the sponge, *Chondrosia reniformis*.
Spirographidin	Spirographin	Obtained from the cartilage and skeletal tissues of the worm, Spirographis.

Similar hyalogens are also described in the vitreous humour, and in hyaline cartilage. These substances resemble one another in their solubility in weak alkalis; from these solutions they are precipitable by acetic acid. They also all yield on treatment with dilute sulphuric acid a reducing sugar with formula $C_6H_{12}O_6$. They are also all insoluble in gastric juice, and soluble in pancreatic juice. They are thus identical in their chief reactions with the mucins. They differ as the various mucins do in minor points. It is, therefore, quite unnecessary to multiply terms or to use the expressions hyalin and hyalogen at all. It is enough to say that from the different tissues just enumerated a mucin is obtainable. Chondroitic acid, the so-called hyalin obtained from hyaline cartilage, differs markedly, however, from ordinary mucin in its low percentage of nitrogen.

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[1] *Virchow's Archiv*, xix. 189.

[2] *Zeit. Biol.* xxii. 261.

[3] The substance of which the nest is composed is secreted from certain glands described by Bernstein (*Journ. Ornithologie*, 1859, p. 111) as being remarkably developed in the swallows during the nest-building season, and atrophying afterwards. Green (*Journ. Physiol.* vi. 40) had previous to Krukenberg pointed out the resemblance of the nest substance to mucin. The word neossin is Mulder's (*Bull. des sc. phys. en Néerlande*, 1838, p. 172).

ADIPOSE TISSUE

Adipose tissue is developed from areolar tissue; the cells of the latter tissue multiply and become filled with minute globules of oil or fat.[1] The globules become larger and coalesce with one another, and these in their turn fuse together until ultimately the cell consists of one large spherical oil-globule, the protoplasmic remains of the cell forming a thin capsule surrounding it; the nucleus is flattened at one side of the cell. These fat-cells become bound together into lobules by the connective-tissue fibres. A very similar process of transformation of protoplasm into fat may occur in other situations; for instance, in the epithelium cells lining the alveoli of the mammary gland during lactation; and in the pathological process known as fatty degeneration in which muscular fibres, liver-cells, and other organic cells are infiltrated with fat-granules and globules, and may in severe cases thus lead to a more or less complete replacement of the protoplasm of the organ by fat. Certain of the liquids of the body, sweat, blood, &c., contain minute quantities of fat (*see* p. 73). It is, however, in adipose tissue that fat is most abundant, and it is with the fat found there that we are in this section particularly concerned. For the formation of adipocere *see* p. 426. For the fat of marrow *see* p. 496.

The Fats of Adipose Tissue

The contents of the fat-cells are fluid during life; the normal temperature of the body (36° C. or 99° F.) being considerably above the melting point (25° C.)[2] of the mixture of fats found in those cells. The fats are three in number, and are called palmitin, stearin, and olein. They differ from one another in chemical composition, and in certain physical characters, such as melting point, and solubilities. On examining fat-cells after death, the fat within them is found to be solid at the ordinary atmospheric temperature, and groups of needle-like crystals may sometimes be detected in their interior. The fat on solidifying has separated in a crystalline form; the fat which has thus crystallised is a mixture of palmitin and stearin, and was formerly called margarin.

Preparation and separation of the fats.—The tissue containing the fat is dried, and then extracted with boiling ether, which dissolves all the fats. The ethereal solution is then evaporated to dryness, and the residue consists of fat.

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[1] Fat may be detected in the cells micro-chemically by the use of osmic acic; this reagent stains fat black.

[2] The fat of the infant body is stated to melt at a higher temperature, as it contains less olein than in the adult.

In order to determine the amount of fat present in a tissue, a portion of the tissue is carefully dried and weighed, and then thoroughly extracted with hot ether; the residue from the ethereal extract is then dried and weighed. From this the percentage amount of fat present can be calculated.

The process of extraction with ether may be most conveniently carried out by means of Drechsel's[1] apparatus. This consists of two flasks; the upper, which is fitted to the lower one, is in connection with a Liebig's condenser, and contains on a fluted filter the finely divided solid from which the fat is to be extracted. In the lower flask is the ether. This is heated on a water-bath; the ether vapour passes up and is condensed in the condenser, and on its way back passes through the upper flask. A constant circulation is thus maintained, and the dissolved fats gradually accumulate in the lower flask.

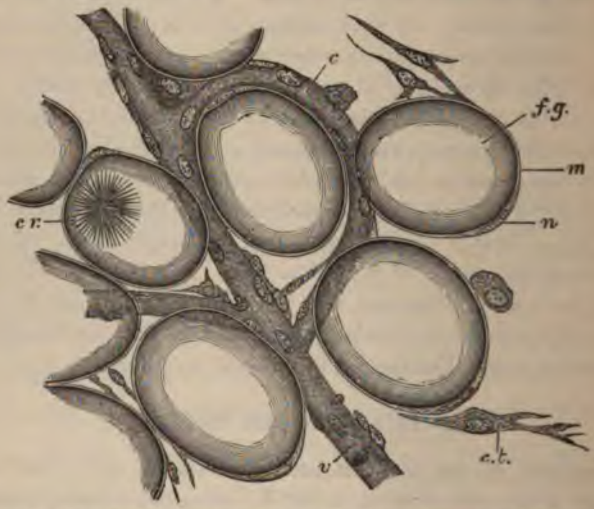

FIG. 76.—A few Fat-cells, one showing so-called margarin crystals, highly magnified (E. A. Schäfer).

The following methods may be employed in the separation of the fats:—Stearin is insoluble in cold ether; fat is repeatedly extracted with cold ether; the residue consists of stearin. Olein is obtained from a mixture of the fats by cooling them to 0° C.; olein alone remains liquid, and may be separated from the others by pressure. By combining these two methods palmitin may be obtained as a residue.

The melting points of the fats may be determined by much the same kind of apparatus as that used in the determination of the heat-coagulation temperature of proteids (*see* p. 119). After having been melted and then allowed to cool, the fat solidifies again, but always a few degrees below that at which it melted.

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[1] *Journ. f. prakt. Chemie*, xv. 350.

The fatty acids may be obtained by dissolving the fats in alcoholic potash, evaporating to dryness, dissolving the residue, which consists of compounds of potash with fatty acids (potash soaps) in water, and finally adding hydrochloric acid ; the fatty acids are thus liberated : these are solid and may be collected on a filter.

The fatty acids may be separated from one another by dissolving them in hot alcohol; this solution is treated with lead acetate, and insoluble lead soaps are thus obtained. Lead oleate is extracted from the mixture by boiling ether ; and lead stearate and palmitate remain undissolved. By adding hydrochoric acid to the ethereal solution of lead oleate, oleic acid is liberated and remains dissolved in the ether. The mixture of lead stearate and palmitate may be also decomposed by hydrochloric acid, and the fatty acids so liberated dissolved in alcohol, and separated by fractional precipitation with barium chloride or acetate ; the stearic acid is precipitated as barium stearate first. On adding more of the barium salt, a mixture of the stearate and palmitate of barium is obtained ; and finally the precipitate consists wholly of barium palmitate ; the successive precipitates are collected on separate filters. An approximate estimation of the composition of a mixture of palmitic and stearic acid (the acids obtained from palmitin and stearin respectively) may be made by means of determining the melting and solidifying points of the mixture. Tables have been constructed which give these particulars with regard to various proportions of these substances when mixed together.[1]

General properties of fats.—They are all soluble in hot alcohol, ether, benzol, carbon disulphide, and chloroform. They all have the physical characteristic known as greasiness. Each is solid below a certain temperature. Above this temperature, known as the melting point, they are fluid. The melting point varies somewhat according to the treatment to which the fat has been subjected.

When mixed with colloid substances in an alkaline solution, fats are broken up into microscopic globules, so that the fluid becomes white like milk, and the suspended fat does not readily rise to the top or separate from the fluid ; such a mixture is known as an *emulsion*. The change known as *saponification* is a chemical change. It occurs when a fat is mixed with certain metallic compounds ; the fat splits into its two components, glycerin and a fatty acid, the latter combining with the metallic base to form what is called a soap. Thus when palmitin is boiled with potash the result is glycerin, and potassium palmitate. The potash and soda soaps are soluble in water; the lead soap is insoluble.

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[1] Heintz, *Poggendorff's Annalen*, xcii. 588.

Stearin	*Palmitin*	*Olein*
Formula :— $C_3H_5(O.C_{18}H_{35}O)_3$.	$C_3H_5(O.C_{16}H_{31}O)_3$.	$C_3H_5(O.C_{18}H_{33}O)_3$.
Melting pt. 53°–66°C.	45°C. (approx.)	0° C.
Solubilities. Nearly insoluble in cold alcohol and ether. Soluble in both when hot	More soluble than stearin in both hot and cold alcohol and ether	Easily soluble in both cold and hot alcohol and ether
Remarks. The chief constituent of the more solid fats (like mutton suet)	More abundant in the adipose tissue of man than stearin	Dissolves all the solid fats, especially at 30° C. or above; it is thus this fat which holds the other two in solution at the temperature of the body. The subcutaneous fat is said to contain more olein than that of internal parts and of marrow
It crystallises from alcohol in brilliant quadrangular plates	It crystallises in fine needles	

Chemical constitution of the fats.—The fats found in adipose tissue are compounds of glycerin or glycerol with fatty acids, and they may be termed glycerides.[1]

The fatty acids form a series of acids derived from the monatomic alcohols by oxidation. Thus to take ordinary ethyl alcohol, C_2H_6O; the first stage in oxidation is the removal of two atoms of hydrogen to form aldehyde C_2H_4O; then on further oxidation these are replaced by one of oxygen to form acetic acid, $C_2H_4O_2$.

A similar acid can be obtained from all the other alcohols. Thus:

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[1] The term hydrocarbon applied by some authors to the fats is wholly incorrect; a hydrocarbon is a compound like marsh gas (CH_4) or olefiant gas (C_2H_4) consisting of hydrogen and carbon only. In spermaceti the fats are not glycerides, but derivatives of cetyl alcohol, $C_{16}H_{33}.OH$, the chief being cetyl palmitate. In Chinese wax (produced by the *Coccus ceriferus*) and in bees' wax there are also no glycerides. The wax is a mixture of ceryl-cerotate $\left.\begin{matrix}C_{27}H_{55}\\C_{27}H_{53}O\end{matrix}\right\}O$, free cerotic acid, $C_{27}H_{54}O$, and melicyl palmitate, a derivative of melicyl alcohol, $C_{30}H_{61}.HO$ (Schorlemmer, *Org. Chem.* p. 174).

From methyl	alcohol,	$CH_3.HO$,	formic	acid,	$CHO.HO$,	is obtained
,, ethyl	,,	$C_2H_5.HO$	acetic	,,	$C_2H_3O.HO$	,,
,, propyl	,,	$C_3H_7.HO$	propionic	,,	$C_3H_5O.HO$	,,
,, butyl	,,	$C_4H_9.HO$	butyric	,,	$C_4H_7O.HO$	,,
,, amyl	,,	$C_5H_{11}.HO$	valeric	,,	$C_5H_9O.HO$	,,

and so on (*see* p. 65).

Or in general terms:

From the alcohol with formula $C_nH_{2n+1}.HO$, the acid with formula $C_nH_{2n-1}O.HO$ is obtained.

This is the series of acids known as the fatty acid series; the sixteenth member in the series has the formula $C_{16}H_{31}O.OH$, and is called *palmitic acid*; the eighteenth has the formula $C_{18}H_{35}O.OH$, and is called *stearic acid*. Each acid, as will be seen, consists of a radicle, $C_nH_{2n-1}O$, united to hydroxyl; and it is these radicles that unite with glycerin to form fats.

Oleic acid, however, is not a member of the fatty acid series proper, but belongs to a somewhat similar series of acids known as the acrylic series, of which the general formula is $C_nH_{2n-3}O.OH$. It is the eighteenth term in the series, and its formula is $C_{18}H_{33}O.OH$ (*see* p. 69).

Glycerin, or *glycerol*, is a triatomic alcohol, $C_3H_5(OH)_3$, i.e. three atoms of hydroxyl united to the radicle glyceryl, C_3H_5; or three atoms of water in which half the hydrogen is replaced by the triatomic radicle, C_3H_5. The hydrogen in the hydroxyl atoms is replaceable by other organic radicles. As an example take the radicle of acetic acid, acetyl (C_2H_3O). The following formulæ represent the derivatives (ethers) that can be obtained by replacing one, two, or all three hydroxyl hydrogen atoms in this way:—

$$\underset{\text{[glycerol]}}{C_3H_5\left\{\begin{array}{l}OH\\OH\\OH\end{array}\right.}\quad\Bigg|\quad\underset{\text{[monoacetin]}}{C_3H_5\left\{\begin{array}{l}OH\\OH\\O.C_2H_3O\end{array}\right.}\quad\Bigg|\quad\underset{\text{[diacetin]}}{C_3H_5\left\{\begin{array}{l}OH\\O.C_2H_3O\\O.C_2H_3O\end{array}\right.}\quad\Bigg|\quad\underset{\text{[triacetin]}}{C_3H_5\left\{\begin{array}{l}O.C_2H_3O\\O.C_2H_3O\\O.C_2H_3O\end{array}\right.}$$

Triacetin is the type of a neutral fat; stearin, palmitin, and olein ought more properly to be called tristearin, tripalmitin, and triolein respectively. Each consists of glycerol in which the three atoms of hydrogen in the hydroxyl are replaced by radicles of the acid.

The following formulæ represent their constitution:—

Acid	Radicle	Formula of glycerol	Fat
Palmitic acid, $C_{16}H_{31}O.OH$	Palmityl, $C_{16}H_{31}O$	—	Palmitin, $C_3H_5(O.C_{16}H_{31}O)_3$
Stearic acid, $C_{18}H_{35}O.OH$	Stearyl, $C_{18}H_{35}O$	$C_3H_5(OH)_3$	Stearin, $C_3H_5(O.C_{18}H_{35}O)_3$
Oleic acid, $C_{18}H_{33}O.OH$	Oleyl, $C_{18}H_{33}O$	—	Olein, $C_3H_5(O.C_{18}H_{33}O)_3$

Decomposition products of the fats.—The fats split up into the substances which we have seen go to build them up.

Under the influence of superheated steam, and in the body under the influence of certain ferments (for instance, the fat-splitting ferment steapsin of the pancreatic juice), a fat combines with water, and splits into glycerol and the fatty acid. Take, as an example, tripalmitin or palmitin, as it is more generally called. The following equation represents what occurs :—

$$\underset{\text{[palmitin]}}{C_3H_5(O.C_{16}H_{31}O)_3}+3H_2O=\underset{\text{[glycerol]}}{C_3H_5(OH)_3}+\underset{\text{[palmitic acid]}}{3C_{16}H_{31}O.OH}$$

In the process of saponification, we have much the same sort of reaction ; the final products are, however, glycerol and the palmitate of the base employed. As an instance take what occurs when tripalmitin is heated with potassium hydroxide ; *mutatis mutandis* similar reactions occur with other fats and other bases ; the equation representing the reaction is as follows :—

$$\underset{\text{[palmitin]}}{C_3H_5(OC_{16}H_{31}O)_3}+3KHO=\underset{\text{[glycerol]}}{C_3H_5(OH_3)}+\underset{\text{[potassium palmitate]}}{3C_{16}H_{31}O.OK}$$

When fats decompose, certain volatile acids are liberated, and these it is which give the characteristic smell to rancid fats. When strongly heated, fats give off a characteristic penetrating odour. This is due to the formation of acrolein (C_3H_4O, the aldehyde of allyl alcohol, C_3H_5OH) from the glycerin.

Glycerin is obtained commercially from fats by decomposing them with superheated steam. It may also be obtained by precipitating the fatty acids as insoluble soaps from a solution of fat by litharge and water ; glycerin remains in solution in the water, and may be freed from lead by a stream of sulphuretted hydrogen.

BONE

Bone, like all the other tissues of the body, consists of water, organic substances, and mineral salts ; it differs from most of the other tissues in the large amount of mineral matter present. The mean percentage of water as estimated by Volkmann is 48 ; Lukjanow[1] gives approximately the same number (46–7 per cent.) ; this is a mean of twenty determinations of pigeons' bones. The general composition of normal undried bone without the separation of marrow or blood is given by Hoppe-Seyler thus :—

---

[1] Lukjanow, *Zeit. physiol. Chem.* xiii. 339. Aeby (*Centralbl. f. d. med. Wissensch.* 1871, No. 14) considers that 11–12 per cent. of the water present is in a state of loose chemical combination, analogous to water of crystallisation.

Water	50·00 per cent.
Fat	15·75 „
Ossein	11·40 „
Bone earth . . .	21·85 „

It may be said roughly that two-thirds of the solids present in bone consist of inorganic matter and one-third of organic substances. Zalesky's analyses are as follows :—

	Human bone	Bone of ox	Bone of guinea-pig
Organic constituents . .	34·56	32·02	34·70
Inorganic „ . .	65·44	67·98	65·30

When a bone is soaked in acid (5 per cent. hydrochloric acid, or a saturated solution of picric acid, &c.), it is but little altered in appearance, but it is soft and flexible and has lost two-thirds of its weight; the inorganic salts have been dissolved out by the acid. The opposite process, the destruction of the organic matter, may be accomplished by heating the bone to a white heat ; the organic matter is thus burnt away, and the bone then appears somewhat whiter than normal, and has lost one third of its weight.

The organic constituents of bone consist of—

a. Ossein.—This is the most abundant of the organic matters in bone. It is identical with collagen (*see* p. 471). By boiling with water it is converted into gelatin.

b. Elastin.—This is present in small quantities only. Some of the perforating fibres and a thin membrane lining the Haversian canals, lacunæ, and canaliculi form the source of this substance in bone.[1]

c. Proteids and nuclein—from the cells.

d. Fat.—This is always present in small quantities, even after the removal of all connective tissue and marrow.

The inorganic constituents of bone are—

a. Calcium phosphate—$Ca_3(PO_4)_2$. This is the most abundant of the mineral matters present in bone.

b. Calcium carbonate -$CaCO_3$.

c. Calcium chloride—$CaCl_2$.

d. Calcium fluoride—$CaFl_2$.

e. Magnesium phosphate—$Mg\ (PO_4)_2$.

f. Small quantities of sulphates and chlorides.

---

[1] This membrane lining the Haversian canals was supposed by Brösicke to be composed of keratin; but H. E. Smith (*Zeit. Biol.* xix. 469) has conclusively shown that this is not the case.

Numerous analyses of the bones of different animals are given in full in Hoppe-Seyler's 'Physiol. Chemie' (p. 105).[1] The total amount and relative proportion of the inorganic constituents is, however, very constant in different animals, and the average from the analyses of Heintz, Recklinghausen, and Zalesky (quoted by Hoppe-Seyler) is as follows:—

(The numbers represent percentages of the total ash)

Ca	PO_4	CO_3	Fl	Mg	Cl
38·49	54·46	6·24	1·28	0·44	0·19

From his own numbers Zalesky has calculated the probable composition of the mineral constituents of bone.

Calcium phosphate	83·889
" carbonate	13·032
Calcium in combination with fluorine, chlorine, &c. .	0·350
Fluorine	0·229
Chlorine	0·183

Hoppe-Seyler believes that the characteristic inorganic ingredient of bone, dentine, and enamel is one which has the same constitution as the mineral apatite. The formula for apatite is:

$$Ca_{10}Fl_2(PO_4)_6.$$

In another variety chlorine takes the place of the fluorine:

$$Ca_{10}Cl_2(PO_4)_6.$$

Very small quantities of these compounds, however, occur in bone; the chief compound is one built on the same plan, in which the radicle CO_3 takes the place of the Fl_2 or Cl_2:

$$Ca_{10}CO_3(PO_4)_6.$$

In other words, if such a compound exists, it is a combination of three molecules of calcium phosphate with one of calcium carbonate:

$$3Ca_3(PO_4)_2 + CaCO_3 = Ca_{10}CO_3(PO_4)_6.$$

During the deposition of earthy matter in tissues like bone and shell the deposit occurs, not in crystals, but in the form of globules and granules. In 1857 George Rainey showed that certain crystalline substances when deposited in viscous solutions assume globular and cell-like forms.[2] These globular bodies are termed calcosphærites by Harting. Ord has shown also how in urine the presence of albumin and other colloid substances influences the crystalline form of urinary sediments, causing the angles of the crystals to be rounded, the molecules arranging themselves not in straight lines, but with a curvilinear disposition.

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[1] A large number of other analyses will be found in Gamgee's *Physiol. Chem.* pp. 278–280, quoted from Frémy, *Ann. de Chim. et de Physique* (3), xliii. 47-107. The general result is approximately the same as that given above. In contrast with what is found in true bone, the analysis of the calcified cartilage of the ray may be given: ash per cent. 30·00; calcium phosphate 27·7; magnesium phosphate trace; calcium carbonate 4·3. Fossil bones also analysed by Frémy show a smaller percentage of organic matter than recent bones; they yield gelatin on boiling.

[2] Rainey, *Quart. Journ. Micros. Science*, 1858. *See* also Ord, *On the Influence of Colloids on Crystalline Form*, London, 1879.

DENTINE, ENAMEL, AND OTHER CALCAREOUS AND SKELETAL STRUCTURES

Dentine consists, like bone, of water (10 per cent.) and solids (90 per cent.). The solids are organic and inorganic. The organic solids are rather less abundant than in bone; they consist of collagen and elastin; the latter is derived from the lining of the dentinal tubules. The inorganic solids are like those in bone. From Aeby's analyses, Hoppe-Seyler calculates that the solid matter of dentine is composed of the following constituents :—

$Ca_{10}CO_3(PO_4)_6$	72·06 per cent.
$MgH(PO_4)$	0·75 „
Organic substances	27·70 „

Enamel.—This is the hardest tissue in the body; in the adult it contains 95–97 per cent. of mineral matter, in the infant 77–84 per cent. Hoppe-Seyler's quantitative analyses give the following mean result :—

$Ca_{10}CO_3(PO_4)_6$	96·00 per cent.
$MgHPO_4$	1·05 „
Organic substances	3·60 „

The inorganic matter thus resembles that in bone and dentine. The organic matter does not yield gelatin; this is interesting in view of the fact that enamel is not of a connective-tissue origin, but is epithelial (epiblastic).

Crusta petrosa, or cement.—This is simply bone both from a histological and chemical point of view.

Scales of fishes.—The scales differ in structure in different groups of fishes: in the Elasmobranchs they are composed of true dentine; the Ganoid scales are covered with a brightly polished plate of enamel; this is very rarely found in the Teleostean fishes, in which the scales are bony; the Dipnoi have horny scales.

Pearls from oysters were analysed and found to consist of calcium carbonate 91·72, animal matter 5·94, and water 2·23 per cent. They are not soluble in vinegar unless pulverised (Harley).[1]

Tortoise-shell.—The shield of the tortoise is firmly fixed to the skeleton: it consists of a layer of epidermis or tortoise-shell composed of horny matter or keratin and a layer of bone beneath.

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[1] *Proc. Roy. Soc.* xliii. 461.

The exo-skeleton of the armadillo is composed of bony plates.

Egg-shells (*see* Eggs). *Shells of invertebrates* (*see* p. 454).

Otoliths.—These concretions, formed in various parts of the auditory organs of all animals, consist chiefly of calcium carbonate in a crystalline form; the crystals are imbedded in mucus.[1]

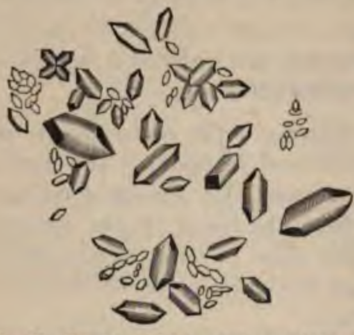

FIG. 77.—Crystals of Calcium Carbonate from an otolith, consisting of small thick columnar crystals, combinations of rhombohedra, and hexagonal prisms.

Phleboliths.—Phleboliths or venous calculi have a tendency to form in veins in which, from dilatation of the coats, the circulation is abnormally slow, as in the veins of the prostate and bladder, and in varicose veins anywhere. They commence, no doubt, as deposits of fibrin, and to this the less soluble salts of the blood adhere, chiefly phosphate of calcium, and in less quantity the sulphates of calcium and potassium. Calcareous deposits in atheromatous arteries have a similar composition.

Brain-sand.—The gritty particles found in the pineal body and in the choroid plexuses are composed of earthy matter (phosphate and carbonate of lime, with a little phosphate of magnesia and ammonia) mixed with organic matter. This substance is not a product of disease, but is present at all ages, and even in the fœtus. Its amount increases with age.[2]

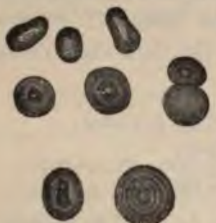

FIG. 78.—Corpora amylacea from human brain.

The corpora amylacea found in the follicles of the pineal gland and pituitary body are coloured brown with iodine, and blue with iodine and sulphuric acid. They are non-nitrogenous, but as they do not yield sugar on treatment with boiling dilute sulphuric acid they are probably not carbohydrate in nature.[3] A colloid substance like that in the thyroid vesicles is sometimes found in the alveoli of the anterior lobe of the pituitary body.

THE FAT OF BONE MARROW

C. Eylert[4] described in ox-bone marrow a new fatty acid melting at 72·5° C., of the formula $C_{21}H_{42}O_2$, which he called medullic acid. As nothing further was discovered as to the properties and salts of this acid, P. Mohr[5] reinvestigated the matter. The fatty acids were separated in the usual way, and the hypothetical acid was found to be nothing but stearic acid; the acids in the marrow fat being present in the following proportions: palmitic acid, 22; stearic acid, 10; and oleic acid, 63 per cent.

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[1] Dahnhardt, 'Endolymphe und Perilymphe,' *Arbeiten d. Kieler physiol. Instit.* p. 18. Barruel, 1838, quoted by Dahnhardt.

[2] Quain's *Anat.* ii. 327.

[3] Hoppe-Seyler, *Physiol. Chem.* p. 689.

[4] Wittstein's *Vierteljahrschrift f. prakt. Pharm.* ix. 330.

[5] *Zeit. Physiol. Chem.* xiv. (1890) 390.

CHAPTER XXIII

THE CONNECTIVE TISSUES IN DISEASE

INTRODUCTORY

THE diseases in which the connective tissues are involved are numerous, but our knowledge of pathological chemistry in this direction is limited.

In actual post-mortem experience chemical methods are comparatively seldom resorted to, as a naked eye or microscopical examination of the organs gives the observer, as a rule, sufficiently complete information of the morbid condition present.

Many of the morbid conditions affecting connective tissue differ from the normal condition in degree rather than in kind. Thus there may be excess of white fibres, producing what is known as fibroid degeneration, cirrhosis, or sclerosis ; or excess of fat may occur as in general obesity ; in this condition widespread fatty degeneration of heart fibres, kidney, liver, &c. may occur in association with increase in the amount of adipose tissue. In another class of cases hypertrophy may be not general, but localised, forming what is known as a tumour ; thus there are bony tumours (exostoses), cartilaginous tumours (enchrondromata), fatty tumours (lipomata), tumours composed of jelly-like connective tissue, as in certain forms of nasal polypi, and so forth. Tumours of this kind are composed of tissue, showing practically no difference from that normally present in the body, and when removed show little or no tendency to recur. There are other new growths of connective-tissue origin which are malignant ; these constitute the numerous class of the sarcomata. A sarcoma, speaking roughly, is composed of embryonic connective tissue in which the cellular elements are especially numerous and active ; and malignancy runs parallel to the activity and rate of growth of these cells. One especially malignant form of sarcoma is that known as melanotic sarcoma. The pigment melanin separated from the tumour has been the subject of several chemical investigations, a brief *résumé* of which will be given.

Another disease which will demand special notice is that known as myxœdema ; and in connection with bone diseases we shall have to consider the chief alterations that occur in rickets, osteomalacia, caries, and necrosis.

Joint diseases are exceedingly numerous. Those of a simple inflammatory nature are accompanied by increase of the synovial fluid, the composition of which we have already considered (*see* p. 351). The more severe the inflammation, the greater is the percentage of organic solids in the effused fluid, and it may even become purulent. In other diseases of joints the cartilage and bone may undergo various pathological changes, and with age hyaline cartilage may become calcified, or even ossified. The so-called loose cartilages that are met with in different articulations consist generally of hard fibrous tissue; the synovial membrane becomes warty and small portions of these pedunculated growths become detached. In some cases, however, they are truly cartilaginous, being portions of articular cartilage that have been chipped off by some injury. The fibrous variety of these foreign bodies appears to be very similar to the loose seed-like structures found in the cysts situated upon the sheaths of tendons which are known as ganglia; in these situations, however, it has been stated that they sometimes consist of lumps of coagulated blood.

A disease which affects cartilage is gout, and we shall have to describe briefly the crystalline deposit of urates found in articular cartilage and other situations in this disease.

In the condition known as dropsy the connective tissues become infiltrated with watery lymph (œdema). Areolar tissue is the most extensively distributed of the tissues, and it is, moreover, continuous throughout the body, and from one region it may be traced without interruption into any other, however distant; thus it is that dropsical fluid, air, blood, or urine effused into the areolar tissues, and even pus, may spread far from the spot where they were first introduced or deposited. The composition of the fluid of subcutaneous œdema will be found on p. 349.

Diseases of marrow might justly be included in diseases of connective tissue; the chief known facts concerning these have, however, been already described in connection with the formation of blood-corpuscles (p. 302).

A rough sketch like the foregoing of the morbid conditions in which the connective tissues are either primarily or secondarily involved is sufficient to indicate the great variety of diseased processes that may occur, and the few points that we have now to take up a little more in detail are those in which chemical research has been instrumental in adding to our knowledge of the pathology of such conditions.

THE PIGMENTS OF MELANOTIC SARCOMATA

The name *melanin* has been hitherto used for the pigments occurring in the eye, hair, skin, in pathological new growths, and also for the decomposition products of chromogens in the urine. We have already considered the black pigments of the eye and skin; the following account of the pigment of melanotic tumours is an abstract of a paper on the subject by Prof. Mörner, of Stockholm.[1] This pigment was first investigated by Heintz, who found that it was soluble in alkalis with difficulty and that it contained no iron. An elementary analysis gave the following figures: C, 53·4; H, 4·02; N, 7·10. Dressler made a similar investigation, and found in the pigment a small quantity of iron. Berdez and Nencki named the pigment phymatorusin; they found it to be insoluble in water, alcohol, and ether; easily soluble in solutions of fixed alkalis or their carbonates, and in ammonia; from such solutions it was precipitable by acids, but was somewhat soluble in excess. The preparation contained carbon, hydrogen, oxygen, nitrogen, and sulphur (in large amount 10·67 per cent.), but no iron, phosphorus, or chlorine. In horses they found in melanotic tumours a pigment with somewhat different properties, which they called hippomelanin.[2] In the urine of patients suffering from melanotic sarcoma a dark pigment has been found; this, according to some, is an excess of the ordinary urine pigment, and, according to others, is the same pigment that occurs in the tumour. It is turned dark brown by the oxidising action of nitric acid, or sometimes by mere exposure to the air. Again, in other cases of these tumours, particles of brown pigment are found in the blood, the corpuscles having the normal shape and colour; similar granules have been occasionally described in the urine and the urinary passages. Mörner's research was undertaken in order to clear up, if possible, some of these doubtful points; the material was supplied from a case of which full clinical and post-mortem records will be found in the paper already referred to. During life the urine showed the peculiar colouration just mentioned; after death the tumour itself was investigated. Its situation was the shoulder, but secondary growths occurred elsewhere. The blood, except for a low percentage of hæmoglobin, was normal. The pigment did not give any absorption bands when examined with the spectroscope, but produced a general dimming, especially near the violet end. By the spectrophotometer the extinction coefficients in different parts of the spectrum were determined. The pigment was also subjected to elementary analysis. It was found to contain iron, which was estimated spectrophotometrically as well as by the usual methods; the spectrophotometric method consists in converting the iron of the ash into ferric thiocyanate, and comparing its extinction coefficients with those obtained from a solution of ferric chloride of known strength similarly treated. The failure of some previous observers to obtain proof of the presence of iron is accounted for by their having used hydrochloric acid in the preparation of the pigment. It is found that this acid dissolves out nine-tenths of the iron from the pigment.

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[1] *Zeit. physiol. Chem.* xi. 66–140. Nencki criticised some of Mörner's statements in *Arch. f. exp. Pathol. und Pharmakol.* xxiv. 27. The difficulties raised by Nencki were fully explained in a subsequent paper by Mörner, *Zeit. physiol. Chem.* xii. 229.

[2] For further particulars *see Arch. f. exp. Path. und Pharmakol.* xxiv. 17; also *Chem. Centralbl.* 1888, p. 587.

Baryta-water caused a precipitate in the urine, which carried down with it some of the pigment; in the filtrate the remainder was precipitated by lead acetate. For the methods which were adopted for separating the pigment from these precipitates and from the tumour the original paper must be consulted. The pigment obtained from all these sources was a brownish amorphous powder when dry. It was partly soluble in acetic acid, and partly insoluble. The following table represents the percentage composition and the relative absorption for the region of wave-length=562, for these different preparations:—

Variety of Pigment	Percentages					Absorption
	C	H	N	S	Fe	
A. Pigment insoluble in acetic acid:—						
1. From the tumour .	55·72	6·00	12·30	7·97	0·072	0·00038
2. From the urine . .	55·76	5·95	12·27	8·65	0·22	0·00034
B. Pigment soluble in acetic acid:—						
1. From the tumour .	—	—	—	5·90	0·21	0·00094
2. From the urine . .	58·07	8·03	11·08	4·75	0·20	0·00085

Although from paucity of materials the analyses are incomplete, the general conclusion seems to be that either two pigments are present, or are produced from a mother substance by the action of the acid. The high percentage of sulphur in the one insoluble in acetic acid, agrees with what Berdez and Nencki found in phymatorusin. An important point brought out is the identity of the tumour pigment with that in the urine; it is probably brought to the urine by the blood, in which feebly alkaline liquid it is slightly soluble. It gives a very different spectrophotometric chart from ordinary urobilin.

Brandl and Pfeiffer[1] have more recently made a similar investigation, and have obtained corroborative results. They consider with Mörner, and in opposition to Nencki, that melanin originates from hæmoglobin.

Melanuria.—This subject (melanin in the urine) has been also investigated by v. Jaksch.[2] He finds that the best reagent for detecting melanin or its precursor, melanogen, in the urine, in cases of melanotic sarcomata, is a very dilute solution of ferric chloride, which gives a black precipitate. These urines also give very markedly the Berlin blue reaction in adding a cyanide, and an alkali, and subsequently an acid. Melanin itself when separated out from the urine does not give the reaction; but it is apparently due to some other substance excreted simultaneously: this substance, whatever it is, appears to be present in traces even in normal urine, and is especially abundant in those urines which yield a large amount of indigo.

MYXŒDEMA[3]

Myxœdema is a well-defined disease, which affects women much more frequently than men, and the subjects are, for the most part, of middle age. In women there appears to be no constant relation between the myxœdematous condition and disease of the generative organs;[4] but pathological and clinical observations both indicate in a most decisive way that the one condition in all cases is a destructive change of the thyroid gland, a delicate fibrous tissue being substituted for the proper glandular structure.

Myxœdema is practically the same disease as that named sporadic cretinism when affecting children; it is also identical with the condition known as cachexia strumipriva, which occurs after removal of the thyroid gland in surgical operations, and lastly it can be artificially produced in certain animals by removal of the thyroid gland.

Affections of movement, speech, sensation, and intellect form a large part of the symptoms of the disease; the most marked morbid condition is an increased bulk of the body, which is due to hypertrophy of the subcutaneous tissues. Interstitial development of fibrous tissue is much less frequently observed in the viscera, and the appearance presented by the new tissue is suggestive of an irritative or inflammatory process.

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[1] *Zeit. Biol.* xxvi. 348.

[2] *Zeit. physiol. Chem.* xiii.

[3] The following account of myxœdema is taken from the report of a committee of the Clinical Society, *Clin. Soc. Trans.* supplement to vol. xxi.

[4] This is a somewhat noteworthy point, in view of the exaggerated importance attached by Landwehr to the co-relation between activity of the female generative organs and an increased formation of substances like mucin, which contain animal gum. *See* p. 480.

In the early stages of this new growth, the microscope reveals an open-textured appearance, probably due to the excess of ground substance, such as is generally met with in young connective tissue; later this tissue is very generally replaced by fat. Dr. Ord,[1] in describing the typical appearances in a case in which death occurred before replacement by adipose tissue had taken place, says: 'The skin did not show the condition of ordinary œdema; during life it was tough and resistant, and after death remained firm on section without exudation of fluid from the cut surfaces: this taken with the microscopic appearances indicates that whatever material filled the unusual interval between the bundles of white fibres had something of a gelatinous consistence.'

The name myxœdema was originally given to this disease on the grounds that one of its most pronounced symptoms was this peculiar œdematous condition, which was not the result of a watery dropsy, but of a swelling of the subcutaneous and other connective tissues, due to an excess of a mucin-yielding intercellular substance. In the disease artificially produced in animals a similar condition was found to be present. The analyses of the amount of mucin present have been made by various observers, who have all adopted, with slight variations, the method which has already been described when we were dealing with the amount of mucin in normal connective tissues (p. 477).

Mucin in myxœdematous tissues (man).—It will be first convenient to recapitulate the chief numerical results obtained with regard to the quantity of mucin present in normal tissues :—

	Percentage of mucin
Skin (infants)	0·766
Skin (adults)	0·385
Connective tissues	0·521
Parotid	traces

The first analyses of mucin in myxœdematous tissues were made by Dr. T. Cranstoun Charles in the case published by Dr. Ord in the 'Medico-Chirurgical Transactions,' lxi. Dr. Charles states (p. 62) that the skin of the feet yielded fifty times more mucin than the skin of healthy people, or those suffering from ordinary œdema. In this case the patient was a female, æt. sixty, and death occurred after the disease had lasted ten years, the patient being still in the swollen condition.

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[1] *Report*, p. 184.

Since then a number of investigations have been made in similar cases, and the observers have given their results more accurately than in the somewhat loose statement made by Charles.

The results may be conveniently drawn up in tabular form :—

Case	Duration of disease	Condition at time of death	Analysis: percentages of mucin				Analyst
			Skin	Tendo Achillis	Cardiac tendons	Other connective tissues and fat	
1. Male æt. 46	4 yrs.(?)	Very fat	—	—	1·9	0·4	Dr. Stevenson
2. Female " 62	—	Wasted	traces	—	—	—	"
3. " " 57	—	Very fat	0·012	—	1·72	traces	"
4. " " 56	32 years	Probably no wasting	0·30–0·38	—	—	—	Dr. Bernays
5. " " 58	3 "	Swollen : much fat	0·36–0·81	—	—	—	"
6. " " 41	16 "	Much fat : possibly some wasting	0·17	—	1·91	—	"
7. " " 44	4 "	Very fat	0·72	—	1·09	0·06–0·20	"
8. Male " 73	—	Swollen	—	1·43	—	—	W. D. Halliburton
9. " " 52	1½ year	Very fat	0·38	—	—	—	
10. " " 47	5 years	Swollen	0·37	—	—	—	"
11. " " —	5 "	Swollen	0·237	—	—	—	"

In several cases other organs, like the brain, liver, and spleen, were examined ; but the results are of little importance, because we have no analyses of the amount of mucin in these organs when in the normal condition ; in organs containing many cellular elements, nuclein and mucin would no doubt be weighed together.

In Case 8, however, the percentage of mucin in the following glands may be stated :—

Parotid gland	0·188
Submaxillary gland	0·159
Pancreas	0·185

In some few cases the blood, and fluids in the serous cavities, which were often increased in amount, were examined.

The general conclusions are as follows :—

Skin.—This tissue was examined in most of the cases, and in ten the analysts stated their results numerically. The lowest percentage of mucin obtained was 0·012, the highest 0·81. In two cases there was an increase of mucin, the percentages being 0·81 and 0·72, the average amount in normal adult human skin being 0·385 per cent. The average of the ten analyses gives a number (0·374 per cent.), which is approximately the same as in normal skin.

Connective tissues.—The tendo Achillis was examined in one case, and the percentage of mucin obtained was 1·42. The cardiac tendons were examined in four cases, and in all there was an increase of mucin, the average number obtained from the four analyses being 1·65 per cent. The average percentage in normal tendinous tissues is 0·521.

Other organs.—The spleen was examined in two cases, and in one gave a large percentage of mucin (2·21). The lungs were examined in the same two cases, and in one case there was a high percentage of mucin (0·72). The liver and brain were examined twice, and the intestine, submaxillary gland, and pancreas have each been examined once; they gave low percentages of mucin, but the amount present in these tissues, when normal, has not been investigated. The parotid, which normally contains only a trace of mucin, gave in the one case in which it was examined a comparatively high percentage of mucin (0·188); this was even higher than the percentage obtained from the submaxillary gland of the same patient (0·159).

Fluids.—The blood has been examined in one case, but no mucin was discoverable in it. It, however, showed very imperfect coagulation, a point in which it resembled the blood of animals in which the disease had been artificially produced. The pericardial, peritoneal, and cerebro-spinal fluids have each been examined in two cases, the pleuritic fluid in one, but in all no mucin was found. There has been no record of mucin having been found in the urine.

It is seen from the foregoing summary of the results of analysis that the increase of mucin, as found by Dr. Charles, has not been found to anything like so great an extent in subsequent cases; nor is the increase so marked as in the experiments on animals. In certain cases this is accounted for by the fact that the patients have not died while in the typical swollen stage, but in the subsequent atrophic period of the disease; and in other cases the subcutaneous connective tissue has become replaced by fat, and in other cases still the analyses are, to a great extent, vitiated by the keeping of the specimens for long periods under alcohol before analysis.

In the case of animals it is easier to avoid all such sources of error.

It is important to remember that the source of mucin in the body is twofold:—

1. It results from the degeneration of the protoplasm of epithelium cells, as in the goblet cells of mucous membranes, and the cells of the acini of mucous glands like the submaxillary. In the myxœdema of human beings this source of mucin has not been to any great extent investigated. The most important analysis bearing on this point is the one analysis of the parotid gland which has been made, and which showed a distinct increase of mucin. This is interesting in connection with Horsley's experiments on monkeys, in which it was shown that in the myxœdema artificially produced in them, the cells of the parotid,

which normally secrete clear saliva, secrete a viscid saliva; by the microscope the cells were found swollen by mucinogen, and by chemical analysis mucin was found to be greatly increased in amount.

2. It forms a constituent part of the ground substance or stroma of connective tissue in which the cells and fibres are embedded. This substance is chemically a muco-albuminous material. One of its constituents is mucin, the other a proteid of the globulin class, which in its reactions resembles the serum-globulin or paraglobulin of the blood. In the chemical investigation of myxœdematous tissues only one of these constituents, viz. the mucin, has been estimated. In new and loose connective tissues the ground substance is present in greater amount than when the fibrous (collagenous) material replaces it in a later stage. This is illustrated by the fact already noted of the higher percentage of mucin in the skin of infants as compared with that of adults. In myxœdema it seems that the swelling is at a certain stage due to the increase of this ground substance, and hence the increased percentage of mucin; but at later stages, when white fibres or fat-cells have permeated it, the increase of mucin is not so marked.

Myxœdema in Animals

The second part of this question deals with the results of the chemical investigation of the tissues of animals. The animals were those in which the thyroid gland had been removed. Some comparative analyses were also performed with the tissues of animals in which the thyroid gland had not been removed. In these experiments the operations were performed by Prof. Horsley at the Brown Institution, and the chemical analyses by myself.

The question which has been the chief subject of chemical investigation is the percentage of mucin in the tissues and fluids of the body.

The first series of analyses made were those which have already been published in Horsley's Brown Lectures ('Brit. Med. Journal,' vol. i. 1885, p. 211). In the tabular form in which they then appeared they illustrate very forcibly the fact that the percentage of mucin is increased in the tissues after thyroidectomy. The increase is not only marked in the connective tissues, but also in the salivary glands. The presence of mucin in large amount in the parotid which normally contains none is especially noteworthy. This chemical result was confirmed by microscopical examination, the cells of the acini simulating those of a mucous gland like the submaxillary. Another important fact is the presence of mucin in the blood in increasing amount as the myxœdematous condition of the animal becomes fully developed (*see* p. 304). It will be noted that the normal percentage of mucin in the skin of monkeys is less than in man. The table, with the additional cases that have been examined since, is as follows :—

Animal	Mucin in parts per 1000					
	Skin and subcutaneous tissues	Tendon	Muscle	Parotid	Sub-maxillary	Blood
I. Normal						
Monkey No. 1 *a* . .	0·89	0·39	0	0	—	0
,, ,, 9 . .	0·9	0·5	0	0	trace	0
II. Abnormal after thyroidectomy						
Monkey No. 1 lived 55 days	3·12	2·55	0	0·72	6·0	0·35
,, , 3 ,, 32 ,,	—	—	—	—	—	trace
,, ,, 5 ,, 49 ,,	2·3	2·4	trace	1·7	3·3	0·8
,, ,, 10 ,, 7 ,,	0·45	0·904	0	trace	·16	trace
,, ,, 6 ,, 29 ,,	1·08	1·5	0	3·08	10·36	,,
,, ,, Y ,, 104 ,,	2·57	—	—	—	—	—
,, ,, Z ,, 21 ,,	2·63	—	—	—	—	—
,, ,, 21[1] ,, 121 ,,	1·4	0·63	—	—	—	—

In some of the above animals the blood was further examined. In monkeys Nos. 1, 3, 5, and 10 clotting of the blood took place very slowly, leading to the formation of a well-marked buffy coat, whereas in normal monkeys the blood coagulates quickly without the formation of a buffy coat. The percentage of proteids in the serum was found to be approximately the same in both normal and myxœdematous monkeys (about 4 to 5 per cent.), the serum-globulin and serum-albumin being present in about equal amount. In one case the temperature of heat-coagulation of the serum-albumin was rather different from the normal. The urine of monkey No. 3 contained a small amount of mucin.[2]

The tissues of a few other animals (pig and donkey), from which the thyroid had been removed, showed no increase of mucin, and the animals themselves showed no typical symptoms of myxœdema, in the same way that monkeys do.

The case of a sheep in which myxœdema occurred after removal of the thyroid is regarded by Horsley and Ord as of great interest; the chemical part of the report in this case ran as follows :—

The blood of the animal was examined twenty-seven days after the operation. No mucin was found in it, and the proteids of the serum were both qualitatively and quantitatively normal (serum-globulin 3·55, serum-albumin 4·13 per cent.) The animal was killed nearly two years afterwards, it having developed myxœdematous symptoms when it was shorn and exposed to cold. It showed no signs of myxœdema before this. The blood, pericardial and cerebro-spinal fluids then contained no mucin. The peritoneal fluid contained a doubtful trace. The urine contained an abundance of mucin. The 'gelatinous' and fatty connective tissue from the anterior triangle of the neck contained 0·9 per cent. of mucin. Although there are no analyses in healthy sheep to compare with this result, it seems to denote a considerable increase in the amount of mucin present, normal adipose tissue in other animals yielding only imponderable traces. The sterno-mastoid muscle and lymphatic glands were also examined from the same animal, and found to contain a trace of mucin.

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[1] Monkey No. 21 was not myxœdematous, being kept at a high temperature, and the percentage of mucin in its tissues is seen to be approximately normal.

[2] The urine in the other cases was not examined.

An impartial examination of all the foregoing results both in man and in animals seems to show that, although the first observers exaggerated the importance of the increase of mucin in the tissues, yet there is some justification for the name myxœdema; the increase of mucin does not appear to be, however, anything peculiar, but simply arises from the fact that all young connective tissue has a smaller proportion of fibres, and a larger proportion of ground substance than fully formed connective tissue. The case of the parotid gland is, however, not explicable in this way, and appears to be one of the most remarkable of the morbid conditions produced by the removal or disease of the thyroid body.

The question arises, how is it that removal of the thyroid or stoppage of its function produces all these remarkable effects? This is an exceedingly difficult question to answer. When the subject had not been fully investigated it was supposed that the thyroid had something to do in carrying out the disintegrative metabolism (katabolism) of mucin, and when the thyroid is removed, mucin accumulates in the tissues. The presence of the colloid material in the alveoli of the normal gland seemed to lend some support to this theory. It is, however, exceedingly difficult to suppose that a gland without a duct could act in this way; it would be necessary to suppose that the blood-stream leaving the gland carried off the excretory products. Such a condition is unknown elsewhere, and as the accumulation of mucin in the tissues is not such a marked symptom as was at one time supposed, the theory just stated cannot any longer be considered tenable.

The view now generally held is that the thyroid gland plays some important part in katabolic processes, and that it is also concerned in hæmatopoiesis (blood-formation), not merely of the corpuscles, but also of certain elements of the plasma (*see* also p. 304). A more exact definition of its functions is still wanting; we know, however, that extensive nervous, degenerative, and irritative changes occur when it is removed or diseased; the metabolic round is broken or interrupted somewhere, and the name given to the group of symptoms produced is myxœdema.

GOUT

Gout is a disease which has been the battle-field of the humoralists and the anti-humoralists, the former attributing the complaint to some morbid condition of the blood and secretions, the latter to some functional disorder or organic change affecting the solid organs of the body. Owing to the researches of Sir A. B. Garrod,[1] gout is now placed among the blood diseases; the poison is urate of soda, and most of the symptoms of the disease are owing to the presence of excess of this substance. We have already considered (pp. 252 and 307) the condition of the blood in this disease, and described the methods adopted for demonstrating the existence of the urate in it.

Different theories are, however, still held to account for the excess of uric acid in the system, some holding that uric acid is formed in excess, and others supposing that the uric acid formed undergoes imperfect oxidation, and so is not removed from the body. The theory of imperfect elimination is supported by the fact that the amount of uric acid in the urine is very small, and that deposits of urates occur especially in those parts which are not very vascular, such as the cartilaginous and fibrous tissues. It is with these collections of urates in the connective tissues that we have here to deal.

The articular cartilages in gout.—The metatarso-phalangeal joint of the great toe is that most frequently affected, and a single attack leaves marks behind which are nearly indelible. A deposit first occurs in the superficial parts of the cartilages in the form of fine crystalline needles, forming a more or less close network, and presenting different degrees of opacity. Subsequently the fibro-cartilages, ligaments, and synovial membranes become involved, the entire surface being rendered more or less irregular and covered with chalky deposits, consisting of urate of soda. The synovial fluid may also contain crystals of the same substance. Owing to the infiltration of the ligaments, the joints become stiffened, and may be ultimately distorted and nodulated.

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[1] Garrod, *A Treatise on Gout and Rheumatic Gout*; art. 'Gout,' in *Reynolds' System of Medicine*. *Med. Chir. Trans.* xxxvii.

The crystals in the cartilage can be readily seen in thin sections with a $\frac{1}{4}$-inch objective, and, as a rule, are arranged in star-like clusters. They doubly refract polarised light.

The presence of uric acid can be readily demonstrated by extracting slices of the cartilage in water of the temperature of 80°–90° C.; the solution is evaporated in a capsule nearly to dryness with a little nitric acid, on exposure of this to the vapour of ammonia, the purple colour of murexide is seen. Or the aqueous extract may be acidified with a little hydrochloric acid, and crystals of uric acid are deposited in a few hours. If the watery solution be evaporated to a syrup without the addition of any acid, bundles of crystalline needles of urate of soda are deposited.

Sometimes similar deposits occur in the arytenoid cartilages, and Cruveilhier found urates deposited in bone itself.

Chalk-stones, or tophi.—Collections of urates forming white chalk-like deposits occur under the skin in various situations, and if excessive lead to distortions and deformities. An opportunity is occasionally afforded of observing the whole train of phenomena from the commencement to the full development of a chalk-stone. This is most readily done in those which appear upon the helix of the ear. A small vesicle first appears between the skin and the fibro-cartilage; its contents are creamy, and present under the microscope the appearance of a clear fluid in which a number of fine crystalline needles are floating. After some months the vesicle assumes the appearance of a white hard bead, closely resembling a pearl, and it may remain as such for years, or it may grow from an increase of the deposit, and in some cases sets up inflammatory and ulcerative processes. The needles in the early stages after the fluid consistency of the deposit has been lost are found aggregated into small bundles, but later it is difficult to separate them, as they adhere and form a closely interlaced mass.

Similar deposits may be found in other situations, such as tendinous aponeuroses of muscles, the sclerotic coat of the eye, and the tarsal cartilages at the angles of the eyes.

White nodules on the ears and other parts containing fat and amorphous granular matter, due to the blockage of the ducts of sebaceous glands, must be carefully distinguished from gouty deposits of urates.

According to Garrod, chalk-stones consist of urate of soda together with small quantities of animal matter and soluble salts derived from the structures in which the concretions have formed. Possibly in some instances, as in a concretion analysed by L'Hérétier, the calcium phosphate found in large amounts was derived from the tissue; in some cases the sodium urate acting as a foreign body may set up inflammation and become infiltrated with calcium phosphate as tubercular matter often does.

Urate of calcium was described as a constituent of chalk-stones by Heintz, and more recently Delépine[1] has found that this salt is more frequently present than is generally supposed. He found it in the urine in cases of gout, and also in the cartilages its typical acicular crystals were present: these give, on being treated with sulphuric acid, a double precipitate of uric acid and calcium sulphate.

The kidney in gout.—A deposit similar to those already described often occurs in the kidney, but the crystals of sodium urate are usually larger. Many of the crystals are situated in the connective tissue between the tubules; some are embedded in the structure of the tubules themselves, and occasionally the tubules are entirely blocked by them, producing white streaks in the pyramids, easily visible to the naked eye (Garrod). Charcot found in some cases that the white matter is partly crystalline, partly amorphous.

RICKETS

Rickets, or rachitis, is a general disorder which attacks children who are subjected to unhealthy hygienic conditions. One of the most marked effects is an affection of the bones during the process of development. In the part of the cartilage where calcification is occurring there is a great proliferation of the cartilage cells; this leads to an enlargement of the epiphyses. The amount of calcareous matter deposited is deficient in the cartilage, and probably under the periosteum also. The bones are thus soft, and bend, especially if the child be allowed to walk; the deformity so produced is rendered permanent by the subsequent complete ossification that occurs.

There is no doubt that insufficient and improper feeding is a very powerful factor in the ætiology of rickets. Various observers have studied the influence of food, rich or poor in earthy salts, upon the composition of bone in animals. Forster[2] observed that the amount of calcium diminished in the bones of dogs when their diet contained little or no lime salts. Zalesky[3] and Weiske[4] in similar experiments obtained altogether negative results. By cutting off the salts of lime from growing animals some have described the production of a condition akin to rickets (Lehmann),[1] while others have not been able to recognise any rachitic symptoms (Tripier,[2] Weiske[3]).

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[1] S. Delépine, *Proc. Physiol. Soc.* 1887, p. ii.

[2] J. Forster, *Zeit. Biol.* xii. 464.

[3] Zalesky, Hoppe-Seyler's *Med. Chem. Untersuchungen*, Heft 1, p. 44.

[4] Weiske, *Zeit. Biol.* viii. 239; x. 410.

Equally contradictory views have been held with regard to the influence of the administration of an increased quantity of calcium salts in the treatment of rickets. The most generally accepted view is, however, that the disease is not due to a diminution of the amount of calcium in the food, but to an inability of the disordered alimentary canal to absorb, and of the disordered bone-forming tissue to appropriate the lime which is present. Treatment should therefore be directed, not to increasing the amount of calcium in the food, but to improving the absorptive and assimilative powers of the child by placing it under appropriate hygienic conditions.

It has been supposed by many writers that lactic acid is produced in the alimentary canal, and that this plays a part as a solvent of calcareous salts deposited in the tissues.

As Gamgee[4] points out, this theory does not rest upon one properly conducted observation, and, like many other crude chemical theories of disease, does not stand the test of even a superficial scientific criticism. He continues as follows :—

'Even assuming that lactic acid were generated, this would necessarily be converted into lactates in the blood. No one has been bold enough to assume that the blood loses its alkaline reaction, for no one could conceive of an acid reaction of the blood being compatible with a prolonged continuance of its functions ; and yet in order that lactic acid could exert any solvent action, it would be necessary that it should exist free in the blood, or that by an unknown chemical decomposition alkaline lactates should be decomposed in the bones.'

The actual alterations that have been found in rachitic bones are as follows :—

1. Their specific gravity falls ; the water and organic matter are increased.
2. The amount of fat is increased ; but not so much as in the mollities ossium.
3. Occasionally they do not yield normal gelatin.

The following analyses of rachitic bones[5] which have been made, show, when compared with those of healthy bone, a very marked contrast. For the purpose of comparison I take some analyses by v. Bibra [1] of the bones of a child æt. two months.

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[1] *Maly's Jahresb.* viii. p. 272.

[2] Léon Tripier, art. 'Rachitisme,' *Dict. encycl. des sciences médicales*, Paris, 1874.

[3] Weiske, *Zeit. Biol.* vii. 179 and 333.

[4] Gamgee, *Physiol. Chem.* p. 283.

[5] Gorup-Besanez, *Lehrbuch*, p. 635.

In 100 parts	Healthy bones of child aged 2 months (v. Bibra)		Rachitic bones		
	Tibia	Ulna	Femur (Marchand)	Tibia (Lehmann)	Humerus (Ragsky)
Inorganic matters	65·32	64·07	20·60	33·64	18·88
Organic matters	34·68	35·93	79·40	66·36	81·12
Calcium phosphate	57·54	56·35	14·78	26·94	15·60
Magnesium phosphate	1·03	1·00	0·80	0·81	
Calcium carbonate	6·02	6·07	3·00	4·88	2·66
Soluble salts	0·73	1·65	1·02	1·08	0·62
Calcium fluoride and loss	—	—	1·00	0·99	81·12
Collagen or ossein	33·86	34·92	72·20	60·14	
Fats	0·82	1·01	7·20	6·22	

MOLLITIES OSSIUM, OR OSTEOMALACIA

This is a disease, occurring in the adult, resembling rickets in causing a softening of the bones. It differs fundamentally from rickets, which affects bones in process of development, in being a morbid process in which the absorption of the salts of fully formed bone takes place. The medullary spaces are much enlarged, and are filled in some cases with red, in others with yellow marrow, and in other cases still with jelly-like connective tissue, such as occurs in the vitreous humour.

Lactic acid, as in rickets, has been supposed to be the *materies morbi*, but the evidence upon which this assertion rests is as unsatisfactory and contradictory as in the case of rickets.[2]

The chief facts derived from examination of the bones in these cases are—

1. The increased proportion of organic matters.
2. The very greatly increased proportion of fat.
3. The corresponding diminution in the mineral matters.
4. In some cases, the bones do not yield gelatin.
5. The bone in some cases is stated to have an acid reaction.

The following analyses of the bones from cases of osteomalacia have been made:—[3]

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[1] Quoted from Charles' *Physiol. and Pathol. Chem.* p. 305.

E. Schmidt, *Annalen d. Chem. u. Pharm.* lxi. 142; Heitzmann, *Maly's Jahresbericht*, iii. 229; Heiss, *Zeit. Biol.* xii. 151.

[3] I am indebted for this table to Gamgee's *Physiol. Chem.* p. 281.

In 100 parts	Bones from patient æt. 40 (Lehmann)		Patient æt. 60 (von Bibra)	Child (Marchand)
	Femur	Rib	Femur	Vertebræ
Organic basis	48·83	50·48	32·54	75·22
Fats	29·18	23·13	4·15	6·12
Soluble salts	0·37	0·63	1·35	1·98
Calcium phosphate	17·36	21·02	53·25	12·56
Calcium carbonate	3·04	3·27	7·49	3·20
Magnesium phosphate	0·23	0·44	1·22	0·92

Another interesting feature of this disease is the presence in the urine of a proteid which has the characters of one of the albumoses. This subject will be referred to again under Urine.

Brittle Bones

The opposite condition to a softening of the bones occasionally occurs; the bones become extraordinarily brittle and break easily. A mild degree of this always occurs with advancing age. The question has been investigated by W. P. Mason,[1] who considers that the increased brittleness is due to the material rather than the structure of the bone; and that it is not due to the increase of cancellous tissue and diminution of the denser portions of the bone, as Frémy supposed.

CARIES AND NECROSIS

A few analyses of carious and necrosed bones have been made; they show that in caries the inorganic constituents are lessened, whereas in necrosis the organic matter is gradually removed. Some few of these analyses are given in the following table :—

In 100 parts	Caries (Becquerel and Rodlier[2])		Necrosis (von Bibra)[3]
	Femur	Metacarpal Bone	
Calcium phosphate Calcium fluoride	51·53	31·36	72·63
Calcium carbonate	5·44	4·07	4·03
Magnesium phosphate	3·43	0·83	1·93
Other salts	0·91	0·30	0·61
Collagen	35·69	59·36	19·58
Fats	3·00	4·08	1·22

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[1] Mason, *Chem. News*, lvi. 157.

[2] *Traité de Chimie pathologique*, p. 546.

[3] Quoted from Gautier's *Chimie appliquée à la physiol. &c.* ii. 543.

CHAPTER XXIV

THE NERVOUS SYSTEM

INTRODUCTORY

The nervous system consists of a central portion (or nerve-centres) and of a peripheral portion, the nerves which conduct nervous impulses either from or to the centres.

The nerves consist of *nerve-fibres* bound together into bundles by means of connective tissue.

The centres consist partly of nerve-fibres (*white matter*) and of the true central portion or *grey matter*.[1] The grey matter consists of *nerve-cells*, and the branches of nerve-cells. The branches of nerve-cells are of two kinds: (1) long unbranched processes which become the central portion (axis-cylinders) of nerve-fibres ; (2) branches which subdivide to a great extent, and probably anastomose, and communicate with the similar branching network of adjoining or more or less distant nerve-cells. The term *neuroglia* is applied by some to the fine network formed by these subdividing processes. The word is, however, more generally used for a supporting framework of branching cells, which are united by their branches, and so form a network throughout both white and grey matter. The cells have in great part lost their nuclei, and thus the resemblance of neuroglia to retiform tissue is very close. By certain methods (Golgi's) of microscopic staining this neuroglia can be distinguished from the fibres of nervous origin. Neuroglia cells are undoubtedly epiblastic, and this tissue thus differs from true retiform tissue, which is mesoblastic. It appears to consist very largely of neuro-keratin.

In addition to the nervous structures proper there are throughout both central and peripheral portions of the nervous system numerous blood vessels and lymphatics. In the case of the centres the grey matter is more vascular than the white.

The reaction of nervous tissues.—The statements that have been made by different observers of the reaction of nervous tissue vary considerably; they, however, all agree in the fact that nervous structures after death are acid. The difficulty in ascertaining the reaction during life is very great, as the nervous structures are permeated by alkaline blood and lymph. The nervous tissues, moreover, of all tissues are those that undergo degenerative processes most readily when their normal blood supply is cut off; so that any attempt to obtain nerve free from blood would not give us living, healthy nerve any longer. Moreover the mere application of reagents in order to test the reaction is generally sufficient to kill the nerve.

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[1] The proportion of grey to white matter in the human brain has been the subject of investigations by Bourgoin (*Recherches chimiques sur le cerveau*, Paris, 1866), and by De Regibus (*Maly's Jahresb.* xiv. 346). Both find the grey matter is the more abundant. De Regibus gives these numbers: for every gramme of grey matter there is 0·75 gr. of white.

The experiments that have been published on this subject are as follows:—

Heidenhain[1] and Gschleidlen[2] state that the normal reaction of the axis-cylinder is alkaline; on death, or after long-continued activity, the reaction becomes acid. The grey matter of the brain is acid even during life. The acid is probably lactic acid. The sympathetic ganglia are neutral or weakly alkaline.

O. Langendorff[3] used the frog in his experiments, as in the cold-blooded animals *post-mortem* changes occur less readily than in the warm-blooded animals. He found that the central nervous system is alkaline in this animal during life, but rapidly becomes acid after exposure or death. He also made similar experiments on rabbits and guinea-pigs, and found that there also the reaction of the brain was alkaline—in newly born animals so strongly, that even after death they did not become acid. On stoppage of the blood-stream, acid accumulates in the brain; but on allowing the blood once more to flow, the accumulated acid is washed away. J. Moleschott and A. Battistini[4] found the brain, spinal cord, and sciatic nerves acid, the grey matter being more strongly acid than the white; on activity the acidity increased, especially in the grey matter. These observers used a very dilute solution of potash and phenolphthalein as indicator. In my own experiments, I have never failed to find an alkaline reaction in fresh brain, cord, or nerve; they, however, rapidly become acid, as a rule, after death.

All observers, however, agree on the most important fact, that acidity, whether present initially or not, increases on activity and on death. The acidity is probably due to lactic acid. This inevitably suggests a comparison between nerve and the closely related tissue, muscle.

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[1] Heidenhain, *Centralbl. f. d. med. Wissensch.* 1868, p. 833.

[2] Gschleidlen, *Pflüger's Archiv*, viii. 171.

[3] O. Langendorff, *Neurolog. Centralbl.* 1885, No. 24; *Centralbl. f d. med. Wissensch.* 1886, No. 25; *Maly's Jahresb.* 1887, p. 323.

[4] *Arch. italiennes*, vol. viii. 90; *Chem. Centralbl.* 1887, p. 1224

The quantity of lactic acid separated from ox brain was 0·5 per 1000; the variety of the acid that is present is not sarco-lactic acid, but the lactic acid of fermentation (Müller) (optically inactive ethidene lactic acid). Kühne supposes this may originate from the inosite in the brain. Müller[1] found 0·8 part per 1000 of inosite and 0·08 part per 1000 of uric acid in ox brain. W. Müller and von Bibra obtained traces of formic acid from brain.

The changes that occur during the activity of nerve are both chemical and physical. The only *known* chemical change that accompanies the transmission of nervous impulses is the increase of acidity just alluded to. The only *known* physical change that accompanies nervous activity is an electrical one. Nervous impulses travel at the rate of 28–33 metres per second; in the case of motor impulses, the nervous excitability is transferred by the agency of the end plates to the muscles,[2] but the rate of the transmission of a muscular impulse (as measured by the wave of contraction in a muscle rendered nerveless by the paralysis of the end plates caused by the administration of curare) is much slower—about three metres per second. Measurements of reflex time, and reaction time, have given us data upon which to calculate the rate of transmission of nerve-impulses through nerve-cells.[3] Incomplete as our knowledge of the essential phenomena of muscular contraction may be, we are still more in the dark with regard to the essential molecular changes that occur on nervous activity. One more point must, however, be again alluded to, namely, the importance of a healthy blood-supply to the nervous organs. Deprivation of oxygen by means of the blood-stream means an abolition of all the higher cerebral functions, such as consciousness and volition; whereas venous blood stimulates the respiratory and other centres in the medulla.

GENERAL COMPOSITION OF NERVOUS STRUCTURES

Water.—The nervous tissues contain a variable amount of water; it is present in larger amount in the grey than in the white matter; in early than adult life; in the brain than in the spinal cord; in the spinal cord than in nerves. These facts are illustrated by the following tables:—

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[1] Müller, *Annalen der Chem. u. Pharm.* ciii. 141. *See* also Strecker, *ibid.* cv. 316.

[2] *See* Kühne, 'Croonian Lecture,' *Proc. Roy. Soc.* vol. xliv (1888), p. 427.

[3] In the frog reflex time varies from 0·008 to 0·015 sec. Reaction time in man varies from 0·125 to ·02 sec.

Portion of nervous system	Percentages of water[1]						
	In fœtus (W)	Age 20–30 (W)	Age 70–94 (W)	(B)	(P)	(M)	(R)
Grey substance . .	87–92	83	84	85	81	81	86
White substance .		69	72	70	68		70
Spinal cord . . .	—	—	—	73–76	—	68	—
Nerves	—	—	—	64–72	—	57	—

Solids.—The solid matters in the brain fall into several classes.

a. Proteids. These comprise about half the solids in grey matter, about one-fourth of those in white matter, and about one-third of those in nerve.

b. Albuminoids. Neurokeratin and nuclein.

c. Phosphorised constituents. Of these the most important are protagon and lecithin, especially in grey matter.

d. Cerebrins. Certain nitrogenous substances of unknown composition.

e. Cholesterin. A monatomic alcohol, especially abundant in white matter.

f. Extractives. Substances that occur in small quantities, such as are found also classified as extractives in muscular tissue (creatine,[2] xanthine,[3] hypoxanthine,[3] inosite, lactic acid, leucine,[4] uric acid, and urea).

g. Gelatin and fat, derived from the adherent connecting tissue.

h. Inorganic salts. The total mineral matter varies according to different writers from 0·1 to 1 per cent. But little is known of the function of the mineral constituents, and they may be here conveniently dismissed altogether with the following abbreviated table from Geoghegan's[5] paper :—

In parts per 1000 of brain

Total ash	K	Na	Mg	Ca	Cl	PO_4	CO_3	SO_4	$Fe(PO_4)_2$
2·9 to 7·1	0·6 to 1·7	0·4 to 1·1	0·0 to 0·07	0·005	0·4	0·9	0·2	0·1	0·01
—	—	—	—	to 0·02	to 1·3	to 2·0	to 0·7	to 0·2	to 0·09

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[1] The above table is constructed from the published observations of Weisbach (W) (*see* Gamgee, *Physiol. Chem.* p. 445), Bernardt (B) (*Ibid.* 446), Petrowsky (P) (*Pflüger's Archiv*, vii. 367), Moleschott (M) (*see* Charles, *Physiol. Chem.* p. 335), and De Regibus (R) (*Maly's Jahresb.* xiv. 346).

[2] According to Müller, creatine is present in human brain, but absent from that of the ox. It was found by Städeler in pigeon's brain (*Journ. prakt. Chem.* lxxii. 256).

[3] Städeler, *Ann. Chem. u. Pharm.* cxvi. 102; Scherer, *Ibid.* cvii. 314.

[4] Müller, *Ibid.* ciii. 131.

[5] *Zeit. physiol. Chem.* i. 330.

The grey matter is stated by Schlossberger to be richer in total ash, but poorer in phosphates, than the white matter; Petrowsky, on the other hand, obtained more phosphoric acid from grey than from white matter.

The following table gives some of the typical quantitative analyses that have been made of the proportion in which the principal solid constituents occur in different nervous structures :—

Portion of nervous system	Proteids	Lecithin	Cholesterin and fat	Cerebrin	Neurokeratin	Other organic matters	Salts
Grey matter of ox brain(Petrowsky)	55·37	17·24	18·68	0·53	6·71		1·45
White matter of ox brain (*ibid.*) . .	24·72	9·90	51·91	9·55	3·34		0·57
Spinal cord (Moleschott)	23·8	75·1					1·1
Human sciatic nerve (Josephine Chevalier[1])	36·8	32·57	12·22	11·30	3·07	4·0	–

After having looked at the nervous tissues as a whole, and before going on to describe in detail the principal organic substances contained in them, it will be next convenient to take the individual histological elements and the facts we know respecting their chemical composition. Here we have, to a large extent, to rely upon the methods of micro-chemistry, which almost necessarily afford us limited information.

Nerve-cells.—These cells vary much in size and shape in different parts of the central nervous system; the body of the cell is protoplasmic, and therefore chiefly proteid in nature. In this way the high percentage of proteids in grey mater is accounted for.

In many nerve-cells masses of a greyish pigment are often present; this pigment does not seem to have been specially investigated, but is no doubt ultimately derived from hæmoglobin like the other pigments of the body. The nerve-cells of the ganglia of the worm *Aphrodite aculeata* are tinged red. This is due to the presence of hæmoglobin.[2] From this fact, and from the fact also of the greater vascularity of grey as compared with white matter, we may assume, as Gamgee says, that respiratory exchanges go on more actively in nerve-cells than in nerve-fibres.

Nerve-cells have always a well-marked *nucleus*. The substance of

[1] *Zeit. physiol. Chem.* x. 97.

[2] Gamgee, *Physiol. Chem.* p. 420.

which it is composed, appears to be one of the class of phosphorised albuminoids known as nucleins; v. Jaksch[1] separated it from the brain, and Geoghegan[2] estimated that the amount present in that organ was 0·14 per cent. Elementary analysis shows very marked discrepancies from the analyses that have been made of nuclein obtained from other sources, and confirms the decision which we have before arrived at (p. 203), of the possibility either of several varieties of nuclein existing, or that nuclein is not a chemical unit, but a mixture of phosphorised substances.

Nerve-fibres vary in size from $\frac{1}{12000}$ to $\frac{1}{1500}$ inch in diameter. Each nerve-fibre consists of three parts: a central portion known as the axis-cylinder, a transparent outer sheath with nuclei, called the primitive sheath, and between the two a white highly refracting substance known as the medullary sheath, or white substance of Schwann, which is interrupted at intervals called the nodes of Ranvier. The axis-cylinder takes origin as a process of a nerve-cell. During its passage through the grey matter it is naked; when it reaches the white matter of the nerve-centres it acquires a medullary sheath; and it is not until it leaves the brain or spinal cord and becomes bound with other nerve-fibres to form a nerve that it receives the outer or primitive sheath. The optic and auditory nerve-fibres, however, are never covered by this outer sheath.

Many nerve-fibres retain both sheaths until they reach their terminations. Others, especially the small nerve-fibres, pass through ganglia (sympathetic chain, semilunar ganglia, &c.), and when they emerge from these have lost their medullary sheath (Gaskell[3]): they are then known as non-medullated nerve-fibres. These are especially concerned in supplying the muscular fibres of viscera and blood vessels.

Nerve-fibres may be classified according to the direction in which they normally transmit impulses into efferent (from nerve-centres to periphery), afferent (from periphery to nerve-centres), and intercentral (from one part of the nerve-centres to another).

Gaskell has more recently classified the efferent nerve-fibres into two sets, according to the effect of the impulses they transmit upon the metabolic processes of the organs they supply. The metabolism or tissue change of a living structure consists of two parts: a building-up process, assimilation or anabolism, and a breaking-down process of the nature of combustion, or katabolism. The nerves the excitation of which produces activity of the organ they supply are those which produce katabolism, and are called katabolic nerves : such nerves are the motor and secretory nerves. The nerves the excitation of which produce a lessening of the activity of the organs they supply are those which allow of building up, or anabolic changes to occur in those organs. They are therefore called anabolic nerves. Such nerves are the inhibitory nerves of the heart, blood-vessels, and viscera.

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[1] v. Jaksch, *Pflüger's Archiv*, xiii. 469.

[2] Geoghegan, *Zeit. physiol. Chem.* i. 330.

[3] Gaskell, *Journ. of Physiol.* vii. 1 *et seq.*

The primitive sheath, or neurilemma, in the case of motor nerves becomes continuous with the sarcolemma of the muscular fibres they supply ; and the chemical characters of both neurilemma and sarcolemma appear to be identical, both consisting of a homogeneous substance of the nature of elastin. It is, however, more soluble in alkalis than the elastin of elastic fibres (*see* p. 405).

The medullary sheath, or white substance of Schwann. This is generally said to consist of myelin. This substance is not, however, a chemical unit, but a mixture of various substances of which complex phosphorised fats like lecithin, with cholesterin and cerebrin, are the chief. During life it is semi-liquid ; after death it solidifies ; in certain pathological conditions it becomes very liquid (Wallerian degeneration). Like the fat of adipose tissue, it reduces solutions of osmic acid, and the deposit of metallic osmium so produced makes it black. This fact is of great value to the histologist.

Kühne and Ewald[1] found that the axis cylinder and white substance of Schwann are covered with a delicate sheath of a horny substance, and that the two sheaths are connected by numerous transverse and oblique fibrils. The rod-like structures described by MacCarthy[2] in the medullary substance are in all probability part of this horny network. The myelin lies in the interstices of the mesh-work.

This horny matter is called *neurokeratin* ; like the keratin of epidermic structures it resists the action of reagents very powerfully. It is found, not only in medullated nerve-fibres, but in grey matter, and we have already come across it in the retina (pp. 457, 459). The chief interest of this material is derived from embryological considerations. Both epidermis and nervous tissues are derived from the same layer of the blastoderm, namely, the epiblast or ectoderm ; both contain at least one chemical substance in common, viz. keratin.

In the crustacea, chitin takes the place of keratin in the epidermal structures, and similarly neurochitin takes the place of neurokeratin in forming a skeletal support to the nerve-fibres.

The following is the method that is adopted for the *preparation of neurokeratin* : Ox's brain is finely divided, washed with water, digested in cold alcohol, fully extracted with ether, dried and powdered. The powder is boiled with alcohol to extract cerebrin; the residue is boiled with water, digested with artificial gastric juice, and then with artificial pancreatic juice. The undigested residue consists chiefly of neurokeratin, and is purified by thorough washing in succession with dilute alkali, acetic acid, alcohol, and ether, and dried.

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[1] *Verhandl. d. naturhist. med. Vereins zu Heidelberg*, vol. i. Heft 5.

[2] MacCarthy, *Quart. J. Mic. Science*, 1876.

Neurokeratin has the same general properties as keratin. It is, however, less easily soluble than keratin in boiling caustic potash and in boiling dilute sulphuric acid. When burnt it emits the characteristic odour of burning horn. When treated with sulphuric acid it yields leucine and tyrosine. Kühne and Chittenden,[1] who have recently investigated the subject, give the following percentage composition for neurokeratin: C, 56·99; H, 7·54; N, 13·15; S, 1·87; O, 20·45. (Compare analyses of keratin, p. 453.) They also made some estimations of the amount of neurokeratin in different parts of the nervous system; they found the percentage in nerve 0·3 to 0·6; in grey matter, 0·3; but in white matter it is much higher, 2·2 to 2·9.

Cerebrin is much more abundant in white matter, and in nerve than in grey matter (*see* table, p. 518); this favours the view that it is one of the constituents of myelin or the white sheath of Schwann.

The axis-cylinder is the long process of a nerve-cell. It is solid during life, and is composed of a mixture of proteids with complex fats. It dissolves in 0·1 per cent. hydrochloric acid and in 10 per cent. sodium chloride solution. It is made up of a number of fibrillæ; this gives the axis-cylinder a longitudinally striated appearance. The axis-cylinder itself and the fibrillæ that result from its subdivision are not stained by osmic acid, but are stained purplish by gold chloride, from the deposit of metallic gold in them. They are stained reddish violet by a solution of copper sulphate in ammonia. The fibrillæ as seen in the terminations of sensory nerves, e.g. in the cornea, often show varicosities, or little swellings, along their course.

The axis-cylinder is the essential part of a nerve-fibre, being the only part which is continuous from one end to the other. Along it nervous impulses are transmitted; the only known chemical change[2] that occurs on activity is the increase of acidity already alluded to (p. 515). The refractive index of the living axis-cylinder is 1·367; this does not alter during activity (Gross[3]).

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[1] *Zeit. Biol.* xxvi. 291.

[2] Helmholtz (*Comptes rend.* lxxxvii. 533), Heidenhain (*Studien physiol. Inst. Breslau*, iv. 250), and Rolleston (*J. Physiol.* xi. 208) were not able to find any rise of temperature accompanying this change. On the death of nerve, however, heat is given off (Rolleston).

[3] Gross, *Pflüger's Archiv*, xlvi. 56.

Development of nerve-fibres.—These are now generally regarded as being epiblastic. The discovery of neurokeratin in them is very distinctly in favour of this view from a chemical standpoint. The axis-cylinder is undoubtedly an enormously long process of a nerve-cell, and grows outwards to the periphery. The formation of the sheaths is, however, still a point upon which difference of opinion prevails. We have seen that the medullary sheath is divided at regular intervals into a series of internodes, each of which possesses a nucleus, and may therefore be looked upon as representing a number of cells wrapped round the axis-cylinder, the primitive sheath being homologous to the cell-membrane. The fatty matter of the medullary sheath may accumulate within the cell as fat does in connective-tissue cells in the development of adipose tissue.[1]

This view of the formation of the sheaths by the linear coalescence of elongated cells appears to gain support from the behaviour of silver nitrate, which produces the appearance of a black line across the fibre at each node, apparently due to the existence of intercellular or cementing material there. At the same time the part of the axis-cylinder that is exposed at the nodes is lightly stained, so that the appearance of a cross at each node is produced. The part of the axis-cylinder which is stained in this way sometimes exhibits transverse striations known as Fromann's lines.

Degeneration of nerve-fibres.—Nasse first noticed in 1839 the breaking up of the white substance of Schwann in the peripheral end of a cut nerve. In 1852 Waller showed that the process depended on the isolation of a nerve-fibre from its nutritive or trophic centre. Since then our knowledge of Wallerian degeneration has been advanced by many researches, notably those of Ranvier.[2] The chief features in this degeneration are a multiplication of the nuclei and a great liquefaction of the myelin. This change occurs simultaneously in the whole length of the nerve-fibre, which is severed from its trophic centre. The myelin collects into irregular drops, which bulge the primitive sheath in parts, and break up the continuity of the axis-cylinder. Ultimately the liquefied myelin penetrates into the connective tissue of the nerve, and is absorbed and removed by the lymphatics.

In the nerve-centres certain tracts of fibres can be readily traced when the grey matter from which they originate is removed or injured, or when they are disconnected from this grey matter, as by a hæmorrhage or other means. The pyramidal tracts are well-known examples of nervous paths, of which we have derived much anatomical information by the Wallerian method. The individual fibres undergo the same changes as those just described in the case of nerve; but in the nerve-centres there is in addition a great overgrowth of connective-tissue neuroglia: this stains very readily with certain histological staining reagents, such as carmine and aniline blue-black, and so the degenerated tracts can be easily traced in sections, even with the naked eye.

The most marked feature of this degeneration is the change in the white substance of Schwann; though spoken of as a fatty degeneration we must remember that myelin is very largely of a fatty nature to begin with, and we have still to wait for a more exact definition of the change in question.

Hoppe-Seyler,[3] in a case of atrophy of the optic nerve, on comparing the diseased with the healthy nerve, found that in the former the substances soluble in ether were diminished, and the amount of gelatin increased, owing to connective-tissue overgrowth.

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[1] Quain's *Anat.* ii. 178, 179.

[2] Ranvier, *Leçons*, 1878; *Compt. rend.* lxxviii.

[3] *Physiol. Chem.* p. 689.

Having thus described the histological elements of the nervous tissues, we must now return to the various chemical materials of which they are composed. Incidentally, as we have gone along, it has been convenient to describe a few of them; we need therefore not return again to nuclein, neurokeratin, the extractives, or the salts. Referring to the list already given of the chief constituents of nervous tissue (p. 517), it will be seen that we have still to describe the proteids, the phosphorised constituents, the cerebrins, and cholesterin.

THE PROTEIDS OF NERVOUS TISSUE

Notwithstanding the quantitative importance of the proteids in nervous tissue, especially in grey matter, comparatively little work has been done on the subject. Most writers are content to allude to them as proteid matter, or to use the term albumin synonymously with proteid. In a recent research by Baumstark,[1] he speaks of the proteids as resembling caseïn. Petrowsky,[2] who made a few definite experiments on the subject, describes :—

1. A globulin : somewhat resembling myosin.
2. An albumin : coagulated at a temperature of 75° C. ; this is especially abundant in the grey matter.

In my own researches on the subject, in which the more recent methods of the separation of proteids by means of fractional heat-coagulation and saturation with various neutral salts were employed, the following are the chief results :—

The proteids present are :—

1. A proteid which, like cell-globulin *α*, coagulates at 45°–47°C.
2. A proteid which, like myosinogen, coagulates at 56° C. This is absent in white matter.
3. A proteid with the properties of cell-globulin, coagulating at 75° C.

These are all globulins ; albumins are absent in fresh brain ; so also are albumoses and peptones.

There is no doubt that the greater part of the proteid matter in nervous tissue is derived from nerve-cells and the axis-cylinders of nerve-fibres.

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[1] Baumstark, *Zeit. physiol. Chem.* ix. 145–150.
[2] Petrowsky, *Pflüger's Archiv*, vii. 370.

THE PHOSPHORISED CONSTITUENTS OF NERVOUS TISSUE

In the year 1865 Liebreich[1] separated from the brain a material he termed *protagon*; he further found that when decomposed by baryta-water it yielded two acids—stearic acid and glycero-phosphoric acid—and a base called choline.

Hoppe-Seyler, and Diaconow[2] working under Hoppe-Seyler's direction, denied the existence of this substance protagon, and considered that it was a mere mechanical mixture of a phosphorised fat called *lecithin*, with a nitrogenous non-phosphorised substance called cerebrin. Lecithin yields the same three decomposition products that were obtained from protagon by Liebreich. Diaconow's elementary analyses were, however, far from convincing.

The subject in this country was taken up by Gamgee and Blankenhorn[3]; and the result of their work has been that Liebreich's discovery has been fully verified. They showed that protagon is a perfectly definite crystalline substance of constant elementary composition. They also showed that even prolonged treatment with alcohol and ether will not extract lecithin from protagon, as alleged by Diaconow. When protagon is digested with alkalis it yields the same decomposition products as lecithin does. Baumstark[4] has since this fully confirmed Gamgee's work.

An elaborate research by Thudichum[5] has led him to the conclusion that there are three groups of phosphorised substances in the brain, which he terms kephalines (very soluble in ether), myelines (far less soluble in ether), and the lecithines (characterised by their extreme instability). In each of these groups there are several members, the empirical formulæ of which have been calculated. Though somewhat indefinite, Thudichum's researches demand a passing notice in this short historical sketch of the chief steps by which our knowledge on this subject has been attained.

We can now pass on to a more detailed consideration of lecithin, protagon, and their products of decomposition.

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[1] Liebreich, *Annalen der Chem. u. Pharm.* cxxxiv. 29.

[2] Diaconow, *Centralbl. f. d. med. Wissensch.* 1868, p. 97.

[3] Gamgee and Blankenhorn, *Journ. of Physiol.* ii. 113. From this paper and from Dr. Gamgee's account of his work in his *Physiol. Chem.* 427 *et seq.* the above description of protagon and lecithin is in great measure taken.

[4] Baumstark, *Zeit. physiol. Chem.* ix. 329.

[5] Thudichum, *Rep. of Med. Officer of Privy Council*, 1874, p. 113 *et seq.*

Protagon

Preparation.—When pounded brain is treated with water, the myelin swells up and is exceedingly difficult to work with. One of the steps in Liebreich's original process for preparing protagon consisted in treating it with water and ether. Gamgee and Blankenhorn found that this part of the operation could be dispensed with, and their mode of preparing protagon is as follows :—

Fresh ox brain, freed from blood and membranes as completely as possible, is digested for many hours in 85 per cent. alcohol at 45° C. The fluid is filtered hot, and the process repeated with the residue so long as the filtrate when cooled to 0° C. deposits a fair amount of white precipitate. This precipitate is collected, and agitated with ether to extract cholesterin. The residue is then dried in an exsiccator. The resulting mass is powdered, moistened with water, digested for many hours with alcohol at 45° C. and filtered hot. The filtrate is allowed to cool gradually, and protagon separates from it in the form of rosettes of microscopic crystals. It may be purified by recrystallisation.

The average percentage composition of this substance is as follows :

Elements	Gamgee and Blankenhorn	Baumstark	Calculated from the formula
C	66·39	66·53	66·45
H	10·69	11·02	10·66
N	2·39	2·70	2·42
P	1·068	1·049	1·07
O	19·462	18·701	19·40

The average numbers in these three sets of analyses are seen to be in remarkably close agreement. The empirical formula calculated by Gamgee and Blankenhorn from their results is $C_{160}H_{308}N_5PO_{35}$.

Alcohol and ether will not dissolve out lecithin from protagon ; it is therefore not a mere mechanical mixture containing lecithin. It, however, yields on treatment with alkalis the same products of decomposition as lecithin does.

The relation of lecithin to protagon is a point which has still to be worked out.

Protagon is accompanied in the brain with substances which may be provisionally termed cerebrins ; but the cerebrin is not merely mixed with lecithin as Hoppe-Seyler supposed.

Lecithin

Lecithin is not merely found in the nervous tissues, but also in the following places :—

1. In yolk of egg. This was first described by Gobley[1] as lecithin. After Liebreich's discovery of protagon, Parke[2] described the chief phosphorised constituent of eggs as protagon also. Hoppe-Seyler[3] and Diaconow[4] subsequently entertained doubts of the existence of protagon, and showed that lecithin was really the compound present.

2. In blood-corpuscles. The discovery of lecithin in the blood-corpuscles ran through the same vicissitudes. Gobley[5] originally described the phosphorised substance as lecithin, Hermann,[6] after Liebreich's discovery, as protagon, and lastly Hoppe-Seyler[7] and Jüdell[8] showed again that it was in reality lecithin.

The view is now generally held, that although protagon is the chief phosphorised constituent of brain, lecithin is the chief phosphorised constituent of egg-yolk and blood-corpuscles.

3. Lecithin is found in small quantities in most organs of the body; in fact, wherever cellular elements exist, and also in certain secretions, semen, bile, milk, &c.

4. Lecithin is largely found in plant tissues.[9] It apparently forms a large constituent of vegetable fats.[10]

Diaconow and Hoppe-Seyler regard lecithin as the chief phosphorised constituent of nervous tissue, and state that it may be dissolved out from protagon by means of ether; a statement which we have already seen Gamgee was unable to corroborate. Gamgee speaks as follows on the subject :—

'Although the brain yields to alcohol phosphorised bodies other than protagon, the latter is much the most abundant of the phosphorised products, and by no action of ether can it be split into lecithin and a non-phosphorised cerebrin; it is, however, possible, and indeed probable, that amongst the phosphorised principles lecithin is to be reckoned. It is indeed apparent from his (Gamgee's) own work, no less than from a careful study of the researches of Thudichum, that the phosphorised ingredients are numerous.'

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[1] Gobley, *Journ. de chimie et de pharm.* xi. 409; xii. 1; xvii. 401; xviii. 107.

[2] Parke, *Med. Chem. Untersuchungen*, Heft 2, p. 213.

[3] Hoppe-Seyler, *Ibid.* Heft 2, p. 215.

[4] Diaconow, *Ibid.* Heft 2, p. 221; *Centralbl. f. d. med. Wiss.* 1868, p. 2.

[5] Gobley, *Journ. de chim. et de pharm.* xxi. 250.

[6] Hermann, *Arch. f. Anat. u. Physiol.* 1866, p. 33.

[7] Hoppe-Seyler, *Med. Chem. Unters.* Heft 1, p. 140.

[8] Jüdell, *Ibid.* Heft 3, p. 386.

[9] E. Heckel and F. Schlagdenhauffen, *Compt. rend.* ciii. 388.

[10] H. Jacobson, *Zeit. physiol. Chem.* xiii. 32.

The question indeed seems to resolve into this: from the chief phosphorised substance in the brain, whatever it is, we can obtain certain products of decomposition; the same products of decomposition can be obtained from lecithin; the substance in the brain is not, however, identical with lecithin, but it must be something very like lecithin; this something very like lecithin we call protagon, and it is possible that lecithin may be contained in the protagon molecule, though it is certainly not free there.

Preparation of lecithin from yolk of egg (Diaconow).—The colour of the yolk is first extracted with ether; the residue is then treated with absolute alcohol, and the alcoholic extract filtered; alcohol is driven off by evaporation, and a solid body with formula $C_{44}H_{90}NPO_9$ is obtained: this is lecithin. Or the lecithin may be precipitated by adding an alcoholic solution of platinum or cadmium chloride; the yellow precipitate so obtained is washed with ether, and the metal separated from it by a stream of sulphuretted hydrogen. The hydrochloride of lecithin so obtained is dissolved in alcohol and ether, shaken with silver oxide, filtered, and the excess of silver removed by a stream of sulphuretted hydrogen; the silver sulphide is filtered off, and lecithin obtained by evaporating the filtrate to dryness.

Properties of lecithin.—Lecithin is a yellowish white, waxy, hygroscopic solid, soluble in ether and in alcohol; it swells and forms ultimately a kind of emulsion with water or saline solutions. When ignited it burns, leaving meta-phosphoric acid as residue. When decomposed it yields glycero-phosphoric acid, stearic acid, and choline. Its most important compounds are those of its hydrochloride, with platinum chloride $(C_{44}H_{90}NPO_9Cl)_2 + PtCl_4$ and with cadmium chloride, which has *mutatis mutandis* the same formula.

Montgomery[1] showed that when water, glycerin, and other reagents were added to some impure lecithin (or protagon, as he termed it, prepared from yolk of egg) on a microscopic slide, snake-like forms shoot out, bending and curling, and even simulating nerve-fibres, the broken-down matter of brain and spinal cord, and occasionally cells. This observation, coupled with that of Rainey on calcium carbonate crystals (p. 494), is interesting, as it indicates that forms similar to those produced in and by living matter may be artificially produced in dead matter.

Constitution of lecithin.—As in the simpler fats found in adipose tissue, we can here start with glycerin.

The formula for glycerin or glycerol is

$$C_3H_5\left\{\begin{matrix}OH\\OH\\OH\end{matrix}\right.$$

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[1] *On the Formation of so-called Cells*, London, 1867.

that is, a molecule of glyceryl C_3H_5 united to three of hydroxyl. The formula for phosphoric acid is

$$(H_2PO_3)HO.$$

If we replace one of the atoms of the hydroxyl hydrogen-atoms of glycerin by the radicle (H_2PO_3) of phosphoric acid we shall obtain

$$C_3H_5\left\{\begin{array}{l}OH\\OH\\O-PO_3H_2\end{array}\right. = C_3H_9PO_6.$$

This is called glycero-phosphoric acid.

If now we replace the other two hydroxyl hydrogens by stearyl $(C_{18}H_{35}O)$, the radicle of stearic acid, we obtain

$$C_3H_5\left\{\begin{array}{l}O.C_{18}H_{35}O\\O.C_{18}H_{35}O = C_{39}H_{77}PO_8.\\O.PO_3H_2\end{array}\right.$$

This is called distearyl-glycero-phosphoric acid. Lecithin is a compound of this acid with an alkaloid called choline.

The formula for choline[1] is

$$C_2H_4\left\{\begin{array}{l}OH\\N(CH_3)_3OH\end{array}\right. = C_5H_{15}NO_2.$$

This substance (*minus* OH) is united to distearyl-glycero-phosphoric acid, taking the place of an atom of hydrogen in that substance; but there is some divergence of opinion as to the exact mode of attachment. Diaconow[2] regards lecithin as a salt, choline being the base which is united to the distearyl-glycero-phosphoric acid. He states in favour of this view that, on shaking an ethereal solution of lecithin with sulphuric acid, the products of the reaction were choline sulphate and distearyl-glycero-phosphoric acid. The following graphic formula will therefore represent Diaconow's view of the constitution of lecithin :—

$$C_3H_5\left\{\begin{array}{l}(C_{18}H_{35}O_2)_2\\O-PO\left\{\begin{array}{l}OH\\O-N(CH_3)_3-C_2H_4OH.\end{array}\right.\end{array}\right.$$

Strecker[3] on the other hand, considers that lecithin is an ether-like combination, the choline being united to the acid by means of the

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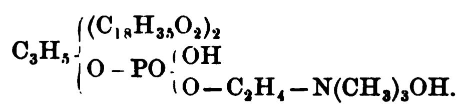
[1] The term neurine is sometimes used synonymously with choline (*see*, however, p. 179).

[2] *Centralbl. f. d. med. Wiss.* 1868.

[3] *Annalen Chem. Pharm.* 1868, cxlviii. p. 77.

oxygen of the hydroxyl; and graphically the formula for lecithin would therefore be



$$C_3H_5\begin{cases}(C_{18}H_{35}O_2)_2\\ O-PO\begin{cases}OH\\ O-C_2H_4-N(CH_3)_3OH.\end{cases}\end{cases}$$

In favour of this latter view Hundeshagen has stated that the choline salt of distearyl-glycero-phosphoric acid prepared synthetically has none of the properties of lecithin. E. Gilson[1] reinvestigated the action of weak sulphuric acid on lecithin. He found that the products of the action were small quantities of glycero-phosphoric acid, another phosphorus-containing compound (? distearyl-glycero-phosphoric acid) in still smaller quantities, and free phosphoric acid; the last named is the most abundant. These results certainly negative Diaconow's theory that choline plays the part of a base in a combination resembling a salt, and we must therefore draw the conclusion that Strecker's is the more correct view to take of the nature of lecithin.

Having now seen the way in which lecithin is built up, it is easy to understand how it is we obtain on its decomposition stearic acid, glycero-phosphoric acid, and choline. The following equation represents what occurs on boiling lecithin with alkaline solutions:—

$$\underset{\text{[lecithin]}}{C_{44}H_{90}NPO_9}+\underset{\text{[water]}}{3H_2O}=\underset{\text{[stearic acid]}}{2C_{18}H_{36}O_2}+\underset{\text{[glycero-phosphoric acid]}}{C_3H_9PO_6}+\underset{\text{[choline]}}{C_5H_{15}NO_2}$$

The acids in the above equation further unite with the alkaline base used to form salts.

Lecithin should more properly be called distearyl-lecithin; other lecithins probably exist in which palmityl, oleyl, or other fatty-acid radicles take the place of the stearyl in the lecithin we have been considering.

The following points may be here added with regard to the chief products of decomposition of lecithin.

Stearic acid has been already considered (p. 491).

Glycero-phosphoric acid.—This may not only be obtained by the decomposition of lecithin or protagon, but may be prepared synthetically from phosphoric acid and glycerin.

It is a syrupy liquid of a sweet-acid taste; its salts, except the lead salt, are soluble in water, and all are insoluble in alcohol. The salts that have been particularly studied are those of barium ($C_3H_7BaPO_6$), of calcium ($C_3H_7CaPO_6.H_2O$ and $C_3H_7CaPO_6.C_3H_9PO_6$), of zinc ($C_3H_7ZnPO_6$), and of lead ($C_3H_7PbPO_6$).

Choline.—This base was first obtained by Strecker[2] from bile, and named by him choline. It may be obtained by decomposing lecithin or protagon by boiling it with baryta-water. The liquid is then filtered, treated with carbonic acid, boiled, filtered, and the filtrate concentrated. A syrupy residue so obtained is extracted with absolute alcohol; the solution is then filtered, faintly acidified with hydrochloric acid, and platinum chloride added. A yellow precipitate consisting of the double salt of choline and platinum chloride is obtained, and choline is obtained free from the metal by the method already described in the case of lecithin (*see* p. 527).

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[1] *Zeit. physiol. Chem.* xii. 585.

[2] Strecker, *Annalen Chem. Pharm.* lxxii. 77.

Choline may also be prepared synthetically from trimethylamine, ethene-oxide, and water [$N(CH_3)_3 + (CH_2)_2O + H_2O = C_5H_{15}NO_2$]; or the hydrochloride may be obtained by heating together ethene-chlorhydrin and trimethylamine [$Cl.CH_2.CH_2.OH + N(CH_3)_3 = N(CH_3)_3(C_2H_4.OH)Cl$] (Wurtz [1]).

Choline is a syrupy liquid, markedly alkaline in reaction, soluble in alcohol and in ether. It forms compounds with hydrochloric, carbonic, and sulphuric acids; its hydrochloride forms compounds with gold chloride and platinum chloride, of which the formulæ are respectively $(C_5H_{14}NOCl) + AuCl_3$ and $(C_5H_{14}NOCl)_2 + PtCl_4$. Choline can be always identified by adding to the substance suspected to contain it gold or platinum chloride, and then estimating the amount of the metal present in the compound so formed. The gold compound of choline contains 44·4 per cent. of gold, the platinum compound 31·9 per cent. of platinum.

When heated, choline splits up into the diatomic alcohol glycol and trimethylamine:—

$$\underset{\text{[choline]}}{C_2H_4\begin{cases}OH\\OH.N(CH_3)_3\end{cases}} = \underset{\text{[glycol]}}{C_2H_4\begin{cases}OH\\OH\end{cases}} + \underset{\text{[trimethylamine]}}{N(CH_3)_3}$$

Physiological importance of choline.—Choline is of great interest and importance, as it is a type of the alkaloids known as ptomaines and leucomaines. These are alkaloids which are formed either after death (ptomaines), as a result of putrefactive changes, or during life (leucomaines), as a result of metabolic processes. Two of the most constantly present are choline and the allied alkaloid neurine (*see* p. 179). Another important alkaloid obtained from poisonous mushrooms, and called muscarine, can be obtained from choline by oxidation. We thus are able to prepare synthetically many of the important organic principles produced in nature by the decomposition of proteids and other complex substances. In Chap. XIII an account of the animal alkaloids will be found.

Choline in small doses produces pyrexia.[2] In larger doses it produces symptoms resembling those of curare poisoning—that is, paralysis due to poisoning of the motor end-plates. Neurine in its physiological action resembles the closely related alkaloid choline, but the former is the more powerful of the two. Both, like muscarine, are antagonistic to atropine with regard to their action on the heart and glandular system.[3]

Fate of lecithin in the body.—Lecithin is largely contained in certain forms of food. In the intestine, pancreatic juice splits it up as it does the simpler fats into its constituents (Bokay [4]); the fatty acid behaves like that obtained from adipose tissue, being partly saponified, partly absorbed as such, and further oxidised in the body to form carbonic acid and water. Glycero-phosphoric acid is also probably absorbed as such, or as a glycero-phosphate. Sotnischewsky[1] found it unaltered in the urine. When mixed with putrefactive organisms it is not decomposed into gaseous compounds. Choline, on the other hand, when mixed with mud containing putrefactive organisms is split up into carbonic acid, methane or marsh gas (CH_4), and ammonia.[2] No doubt a similar decomposition is produced by the bacteria of the intestine, and so the poisonous action of choline is obviated. We are still ignorant of what happens to the lecithin or protagon of the brain. A supposed increase of the output of phosphates during mental activity has never been fully proved (Hoppe-Seyler[3]).

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[1] Wurtz, *Annal. Chem. Pharm.* Sup. vi. 116 and 197.

[2] Ott and Collmar, *Journ. Physiol.* viii. 218.

[3] Cervello, *Arch. ital. biol.* vii. 232; *Chem. Centralbl.* 1887, p. 1150.

[4] Bokay, *Zeit. physiol. Chem.* i. 157.

CHOLESTERIN

This substance is contained, not only in nervous tissue, but also in blood-corpuscles, in bile, and elsewhere. In nervous tissue it appears to be an especially abundant constituent of myelin or the white substance of the medullary sheath.

Preparation from brain or spinal cord.—The tissue is first dehydrated by cold alcohol, then finely divided and extracted with boiling alcohol. The alcoholic solution is filtered hot, and cooled. A deposit occurs which consists of protagon and other phosphorised constituents, cerebrin, and cholesterin. From it the cholesterin is dissolved out by ether, and the ether distilled off. To get rid of adherent traces of lecithin, the residue is heated for an hour with alcoholic potash : this decomposes the lecithin, and the residue obtained by evaporating to dryness is dissolved in a mixture of alcohol and ether ; from this solution cholesterin crystallises out as its solvents evaporate off.

Cholesterin is obtained readily from gallstones by simply extracting them with boiling alcohol, and treating with alcoholic potash to free it from extraneous matter.

Properties of cholesterin.—It is freely soluble in hot or cold ether, glycerin, petroleum, benzol, and solutions of bile salts, in hot alcohol and in chloroform. From anhydrous ether or chloroform it separates in the form of needles containing no water of crystallisation ; from alcohol or ether containing water it separates in the form of rhombic tables ($C_{26}H_{44}O+H_2O$) ; these are easily recognised under the microscope (*see* fig. 79).

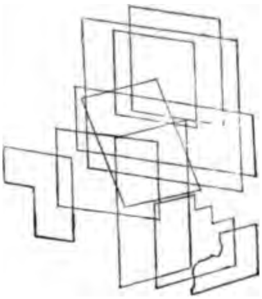

FIG. 79.—Crystals of Cholesterin.

Dry cholesterin melts at 145°, distils *in vacuo* at 360° C. Its specific rotatory power $(\alpha)_D = -31{\cdot}6°$. Its specific gravity is 1·046. It may be recognised by the following colour tests :—

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[1] Sotnischewsky, *Zeit. physiol. Chem.* iv. 215.

[2] K. Hasebroek, *Ibid.* xii. 148.

[3] *Physiol. Chem.* p. 688.

(1) With iodine and concentrated sulphuric acid the crystals give a play of blue, red, and green.

(2) Heated with sulphuric acid and water (5 : 1) the edges of the crystals turn red. These two tests can be watched under the microscope.

(3) A solution of cholesterin in chloroform shaken with an equal amount of concentrated sulphuric acid turns red, and ultimately purple, the subjacent acid acquiring a green fluorescence (Salkowski).

Chemical constitution and derivatives.

Cholesterin is a monatomic alcohol, $\left.\begin{matrix}C_{26}H_{43}\\H\end{matrix}\right\}O$.

It forms a compound with bromine ($C_{26}H_{44}O.Br_2$) ; the compounds of the radicle cholesteryl ($C_{26}H_{43}$) that have been examined are the chloride ($C_{26}H_{43}Cl$) and the amide ($C_{26}H_{43}NH_2$).

Cholesterin yields, on treatment with hot nitric acid, cholesteric acid, $C_8H_{10}O_5$ (Witthaus), and on oxidation, by means of chromic acid, it yields oxycholic acid, $C_{24}H_{40}O_6$. By oxidation with potassium permanganate three acids are obtained, viz. β-cholesteric, $C_{26}H_{42}O_4$, oxycholesteric, $C_{26}H_{42}O_5$, and dioxycholesteric, $C_{26}H_{42}O_6$ (Latschinoff).

Physiological importance of cholesterin.—Cholesterin is very widely distributed in the body (nervous system, blood-corpuscles, yolk of egg, semen, spleen, milk, bile, fæces, &c.) It occurs in excess in certain pathological conditions, e.g. in gall-stones, in atheromatous, cancerous, and tubercular deposits ; in cataract and in certain degenerative diseases of the retina glistening crystals of cholesterin may be often seen with the ophthalmoscope ; crystals of cholesterin are often found floating about in ovarian fluids, less frequently in ascitic and pleuritic fluids. It is present, to a large extent, in the seeds and oils of certain plants—cereals and pulses &c. A substance very like cholesterin (isocholesterin) was prepared by Schultze from sheep's wool ; the vegetable cholesterins have been named paracholesterin by Reinke and Rodewald,[1] and phytosterin by Hesse.[2] These differently named compounds differ slightly in melting point, specific rotatory power, &c. but they are apparently all isomeric.

'The mode of origin of cholesterin in the body has not been clearly made out. Whether it is formed in the tissues generally, in the blood, or in the liver is not known, nor has it been determined conclusively that it is derived from albuminous or nervous matter. It is also doubtful if we can regard it as a waste substance of no use in the body, as its presence in the blood-corpuscles, in nervous matter, in the egg, and in vegetable grains points to a possible function of a histogenetic or tissue-forming character' (McKendrick).[1]

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[1] *Journ. Chem. Soc. Abstracts*, 1881, p. 753.

[2] *Annalen Chem. Pharm.* cxcii. 179. *See* also Jacobson's paper on vegetable fats, *Zeit. physiol. Chem.* xiii. 32.

Determination of Cholesterin, Lecithin, and Fats (*Hoppe-Seyler*)

The method with slight variations is applicable to estimations of cholesterin and lecithin whenever they occur in blood, brain, &c.

A known volume of the liquid (20 to 50 c.c.) or a known weight of the solid is treated with large excess of absolute alcohol; the insoluble residue is again extracted and washed with alcohol, and finally extracted with a mixture of alcohol and ether. The mixed extracts and washings are evaporated to dryness on the water-bath. The residue is dissolved in ether, again evaporated to dryness, and weighed. The combined weight of cholesterin, lecithin, cerebrin, and fats is thus obtained. The residue is then treated with alcoholic potash, and heated on the water-bath till all the alcohol is driven off. The residue contains caustic potash, cholesterin, and the products of decomposition of fats (glycerin and soaps), and of lecithin (choline, glycero-phosphoric acid, &c.). To this water is added, and ether is agitated repeatedly with the mixture. The ethereal solution is evaporated to dryness and weighed; this gives the weight of the cholesterin.

The cholesterin having been removed by the ether, the watery solution is evaporated to dryness and fused with sodium hydrate and pure nitre. The fused mass is dissolved in water, and to it an excess of nitric acid and then ammonium molybdate added; the mixture is allowed to stand twelve hours, the precipitate is dissolved in ammonia, and the phosphates again precipitated by magnesia mixture. This precipitate is washed, dried, ignited, and weighed as magnesium pyrophosphate, 100 parts of which correspond to 764·5 parts of lecithin. The weights of cholesterin and lecithin having been thus obtained, the weight of the fats and cerebrin is obtained by difference.

The cerebrin is subsequently estimated in another portion of brain substance.

THE CEREBRINS

These form a group of ill-defined, nitrogenous substances existing in the white substance of nervous tissue, and it is said also in yolk of egg, pus-corpuscles and spleen.[2]

Müller[3] obtained cerebrin by rubbing brain up with baryta-water so as to form a milky fluid; this is boiled and the resulting coagulum is extracted with boiling alcohol; on cooling, the alcoholic solution deposits cerebrin and cholesterin. The latter is removed by ether, and the former is purified by repeated crystallisation from boiling alcohol.

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[1] *Physiology*, i. 147.

[2] Hoppe-Seyler's *Physiol. Chem.* pp. 720, 788. The cerebrin in the spleen is doubtless obtained from the white corpuscles contained in that organ.

[3] Müller, *Annalen Chem. Pharm.* ciii. 131; cv. 361.

According to Müller, its formula is $C_{17}H_{33}NO_3$; according to Parcus,[1] $C_{80}H_{160}N_2O_{15}$. Parcus also obtained two other similar substances with different formulæ. Adopting a slightly different *modus operandi*, Geoghegan[2] obtained a substance with the formula $C_{57}H_{110}N_2O_{23}$. Thudichum[3] obtained three nitrogenous substances, which may be classified as cerebrins, which he has named cerebrin ($C_{34}H_{66}N_2O_8$), phrenosine ($C_{41}H_{67}NO_8$), and kerasene ($C_{46}H_{91}NO_9$). Gamgee[3] found that whilst protagon cannot be separated by the action of solvents into lecithin, and a non-phosphorised substance cerebrin, yet such non-phosphorised substances do exist by its side in the brain, and one which he terms pseudo-cerebrin ($C_{44}H_{92}NO_8$) can be obtained from protagon by the action of caustic baryta. The above facts show that there are probably several cerebrins, but that our present knowledge of these non-phosphorised, nitrogenous constituents of the brain is most incomplete.

The cerebrins are like mucin in being nitrogenous glucosides; when boiled with acids they yield a lævorotatory, unfermentable sugar (Liebreich,[4] Diaconow, Otto,[5] Geoghegan,[6] Thudichum[7]). This sugar is galactose (Thierfelder,[8] Brown, and Morris[9]).

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[1] Parcus, *Journ. f. prakt. Chem.* cxxxii. 310.

[2] Geoghegan, *Zeit. physiol. Chem.* iii. 332.

[3] *Loc. cit.* [4] *Virchow's Arch.* xxxix. 183. [5] *Ibid.* xli. 272.

[6] Geoghegan stated that the substance which reduced alkaline solutions of cupric salts had the formula $C_{22}H_{42}O_5$; he named it *cetylid*. By fusing it with a caustic alkali, palmitic acid was obtained. There is, however, no doubt that cetylid was a mixture of a sugar with other decomposition products of cerebrin.

[7] *Journ. f. prakt. Chem.* xxv. 23.

[8] *Zeit. physiol. Chem.* xiv. 209. [9] *Proc. Chem. Soc.* London, 1889, p. 167.

CHAPTER XXV

THE ORGANS OF THE BODY

THE animal body is built of a number of constituent parts called organs. Each organ has a special function. The functions of different organs are, however, interrelated more or less closely. Those of which the functions are more closely connected to one another are grouped together into sets of organs or systems. We have thus the circulatory, respiratory, alimentary, and other systems.

The organs, moreover, are built up of certain elementary textures or tissues, and in the preceding chapters we have been dealing with the chemical physiology of these tissues. In considering the chemistry of the organs, we shall find each to consist of several of the tissues, and therefore containing the substances found in those constituent tissues.

Many of the organs we have now to consider may therefore be dismissed in a few words; others, such as the liver, will demand more detailed study; and others again which form secretions will be only studied in part. In this chapter the chemical constituents found in the organs themselves will alone be considered; the secretions they form can be more conveniently studied in relation to alimentation, nutrition, and excretion.

Relation of water and solids in various organs.—The following analyses, most of them by Oidtmann,[1] of some of the organs which we shall consider give the relative normal amount of water, organic matter, and mineral matter in each :—

Organs	Water	Organic substances	Inorganic substances
Liver (child)	74·14	24·78	1·07
Liver (old woman) . . .	80·63	18·65	0·71
Spleen	70·77	23·30	0·5–0·9
Thymus (dog).	80·7	19·2	0·2
Thymus (calf)	77	21	2·0
Thyroid (child)	77·2	22·3	0·5
Thyroid (old woman) . .	82·2	17·6	0·9
Suprarenal body. . . .	80·03	19·88	0·09
Kidney (child)	77·82	21·47	0·71
Kidney (old woman) . .	81·09	17·92	0·1
Lung	79·6	19·8	0·6
Testis (Miescher) . . .	75·0	25·0	

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[1] *Preisschrift* Würzburg, 1858.

The relation of water to solids was determined in a large number of the organs and tissues of twenty normal pigeons by S. M. Lukjanow.[1] These were compared with similar observations on twenty pigeons from which food and water had been withheld for some time. The chief conclusions to be drawn from the exhaustive tables of results are as follows :—

Organs and tissues of the starving animals only showed important changes in the relation of solid to water when the total body-weight was diminished by 34 per cent. and the animal had taken no solid or liquid food for 133 hours. The relation in some organs (heart, kidneys, thorax muscles, alimentary tract, blood, brain, and lungs) undergoes little or no change; in others (thigh muscles, bones) the water is increased ; while in a third category (spleen, pancreas, liver) the water is diminished. Sex and initial weight are apparently factors that have no influence.

The following table gives the average percentages of water and solids in the organs as found by Lukjanow (*see* also p. 58) :—

	Blood	Brain	Thorax muscles	Liver	Pancreas	Alimentary walls	Spleen	Kidneys	Heart	Lungs	Thigh muscles	Thigh bones
Normal												
Water. . .	77·07	80·16	72·95	74·27	75·29	76·53	78·90	77·41	77·14	78·08	74·86	46·68
Solids. . .	23·93	19·84	27·05	25·73	24·71	23·47	21·10	22·59	22·86	21·92	25·14	53·32
In inanition—												
Water. . .	77·44	79·78	73·25	72·19	74·08	76·21	78·22	77·55	77·05	77·67	76·57	51·32
Solids. . .	22·56	20·22	26·75	27·81	25·92	23·79	21·78	22·45	22·95	22·33	23·43	48·68

THE LIVER

The liver may be considered as a mass of epithelial cells prevaded in all directions by blood-vessels and bile-vessels. In most forms of epithelial tissue, the constituent cells are spread out in layers to form a membranous investment or lining of some organ. But in the liver the cells are collected together into lobules, the whole being bound together by means of connective tissue. The liver is one of the largest masses of cells in the body, and is larger in proportion in the embryo than in the adult; these cells perform many important functions. They are formed from the same embryonic layer, the hypoblast, as that from which the cells that line the alimentary canal are formed, and the function of the liver is intimately connected with the processes of alimentation.

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[1] *Zeit. physiol. Chem.* xiii. 339.

The liver receives a supply of arterial blood by the hepatic artery. This appears to be concerned chiefly in supplying the supporting connective tissue of the organ. The chief supply of blood to the liver is venous blood; this comes *via* the portal vein, formed by the union of the mesenteric and splenic veins; the portal vein breaks up into capillaries after the manner of an artery; the blood leaves the liver by means of the hepatic veins, which open into the vena cava inferior. During digestion the portal vein carries to the liver certain products of digestion absorbed from the alimentary canal; this is taken from the blood by the liver-cells, and stored up there chiefly as glycogen. This is again given out as necessity arises, probably in the form of a soluble sugar, and leaves the liver by the hepatic vein. The storage capacity of the liver led Claude Bernard to compare its function with that of the tuber of a potato plant; the tuber stores up carbohydrate in the form of starch, receiving it in a soluble form from the leaves, where it is formed, and giving it out again as a soluble carbohydrate.

The important secretion called bile is also formed by the liver-cells. This will be considered in connection with digestion.

The liver-cells have lastly a most important action in producing urea and uric acid, and other products of nitrogenous metabolism, which ultimately pass into the urine, and will be considered in connection with that secretion.

Chemical Composition of the Liver Substance

The fresh liver is alkaline in reaction, but after death soon becomes acid, and this, as in so many cases, is due to the development of sarcolactic acid.

The number of organic substances occurring in the liver is very numerous. There are proteids and nuclein contained in the protoplasm and nucleus respectively of the hepatic cells themselves; there are substances, like glycogen, sugar, and fat, which are stored up by the liver-cells, or produced from stored-up substances; there are certain constituents, such as gelatin and mucin, derived from the connective-tissue framework; blood and bile may also be found if means have not been taken to remove these previously. There are also extractive matters like xanthine, hypoxanthine, and uric acid; and lastly a certain small proportion of inorganic constituents.

The proportion of water present is roughly the same as in muscular tissue, viz. 75 per cent. The following numbers are given by v. Bibra:[1]

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[1] V. Bibra, *Chemische Fragmente über die Leber*, 1849.

Water	76·17
Insoluble tissues	9·44
Proteids	2·40
Gelatin	3·37
Extractives	2·40
Fats	2·50

Oidtmann[1] found 1·1 per cent. of inorganic material of which potassium phosphate, as in most solid organs of the body, was the most abundant. His numbers are as follows :—Potash, 25·17 ; soda, 14·17 ; lime, 3·62 ; magnesia, 0·19 ; iron oxide, 2·75 ; phosphoric acid, 43·37 ; sulphuric acid, 0·91 ; silicic acid, 0·27 ; chlorine, 2·5 ; traces of manganese, lead, and copper. Calcareous deposits, consisting chiefly of calcium phosphate and carbonate, may occasionally be found in the liver.

The Proteids of the Liver-cells

This has been the subject of an interesting research by P. Plósz.[2] Fresh liver substance was found to be alkaline, after death it became first neutral and then acid. The liver at the same time became harder and less transparent, and these changes are all attributed to a condition resembling the *rigor mortis* of muscle. The liver was rapidly washed free from blood and bile by means of a stream of ice-cold salt solution (0·75 per cent. sodium chloride), cut into small pieces by means of cooled knives, frozen, and the pieces subjected to pressure. As the pieces thawed, an alkaline juice was expressed from them, which may be termed *liver-plasma*, as it appeared to be analogous with Kühne's muscle-plasma (*see* p. 406). Here, however, resemblance ceased, as clotting never occurred in the plasma ; myosin is therefore not present in liver-plasma. The liver-plasma contained in solution a proteid coagulating at a temperature of 45° C. (in this it resembles muscle-plasma) and a nucleo-albumin ; in the cells from which the juice had been expressed, a globulin which is more difficult of solution than the two just mentioned. The liver-plasma also contained glycogen and small quantities of sugar. A more thorough investigation of the proteids was made by extracting the proteids from the liver-cells by means of saline solutions ; 0·75 per cent. sodium chloride, 10 per cent. sodium chloride, and solutions of sodium sulphate and other salts were employed to dissolve the proteids. The proteids present were :—

1. A proteid coagulating at 45° C. ; wholly soluble on gastric digestion.

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[1] Oidtmann, 'Die anorganischen Bestandtheile der Leber,' *Preisschrift*, Würzburg, 1858.

[2] *Pflüger's Archiv*, vii. 371.

2. A nucleo-albumin coagulating at 70° C., yielding an insoluble residue of nuclein on gastric digestion.

3. A globulin coagulating at 75° C. This was most readily extracted with a 10 per cent. sodium chloride solution. It also was wholly digested by gastric juice.

4. Alkali-albumin.

5. The nuclei contained nuclein.

I have repeated Plósz' experiments with certain slight variations, and find that saline solutions extract the following proteids from the liver-cells :—[1]

1. A globulin coagulating at 45° C.
2. A globulin coagulating at 56° C.
3. A globulin coagulating at 70° to 75° C.
4. An albumin coagulating at 70° to 73° C.

No. 1 is probably identical with what I have termed cell-globulin α (*see* p. 260).

No. 2 resembles myosinogen in its coagulation-temperature, but like Plósz I have failed to find any further evidence of myosin. I should propose the name hepato-globulin for this substance.

No. 3 is cell-globulin (*see* p. 260).

No. 4 is cell-albumin, but is present in the merest traces, and may be practically absent in many cases.

I have failed hitherto to obtain any evidence of nucleo-albumins. I am inclined to regard the hardening that occurs in the liver after death, and which is very slight, as not being comparable to the *rigor mortis* of muscle, but is more probably due to the solidification of the fat in the cells, which during life is liquid. It is, however, possible, as Plósz suggests, that if coagulation does occur in the cells with the formation of a myosin-like clot, this takes place so rapidly that our present methods do not enable us to separate its precursor from the cells.

The Glycogen of the Liver

The glycogen of the liver-cells can be frequently demonstrated in them micro-chemically by means of iodine; glycogen is stained a reddish-brown colour by this reagent; it occurs in globules or in irregular amorphous masses within the cells (Heidenhain[2]), and when abundant reduces the protoplasm of the cell to the condition of an open network which becomes very distinct after solution of the glycogen (fig. 80).

Preparation of glycogen from the liver.—A rabbit is killed three or four hours after a hearty meal of carrots. The blood is washed from the liver by passing a stream of salt solution through the vessels, a cannula is inserted into the portal vein for this purpose; another cannula is placed in the vena cava inferior and the mixture of blood and saline solution which comes from the liver can be collected. The first portions that come through contain sugar. When the organ is rendered colourless, and the salt solution that leaves the liver is no longer deeply tinged with blood, the liver is removed and plunged into boiling water acidulated with a little acetic acid. A certain amount of glycogen is in this way extracted, and the proteids of the liver-cells are coagulated. Any ferment too which may be present, and which would convert the glycogen into sugar, is destroyed. The pieces of liver thus scalded are then thoroughly extracted with boiling water, and filtered off. The extract is very opalescent, and contains a trace of proteid, which may be precipitated by means of a little hydrochloric acid and potassio-mercuric iodide, and filtered off. The filtrate is concentrated, and excess of rectified spirit added. This precipitates the glycogen as a white amorphous powder, which is collected, washed with ether and absolute alcohol, and dried (Brücke [1]).

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[1] *Proc. Physiol. Soc.* 1890, p. 9.

[2] Heidenhain, *Hermann's Handbuch*, 1880.

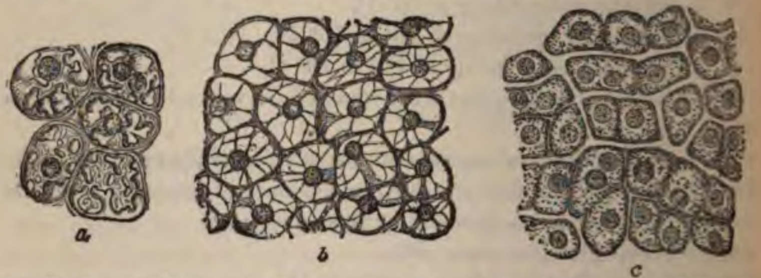

FIG. 80.—Hepatic Cells from the Liver of a Dog, fourteen hours after a full meal (Heidenhain): *a*, with glycogenic deposit; *b* and *c*, after its solution. In *c* the network which remains is finer than in *b*, and imparts a somewhat granular appearance to the cells. The external layer of the protoplasm contains no glycogen.

Külz [2] recommends a dilute solution of potash instead of water for extracting the glycogen from the liver.

If a quantitative estimation is to be made, a weighed quantity of liver must be taken in the first instance, and it must be repeatedly extracted until no more glycogen passes into solution. The dried glycogen obtained from all the extracts mixed together is also weighed; or the glycogen may be converted into sugar by boiling with sulphuric acid and then estimated polarimetrically (Külz), or by means of Fehling's solution.

In the liver the glycogen is equally distributed throughout; it is therefore only necessary to use a small portion of the liver for quantitative estimations (Cramer[1]).

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[1] Brücke, *Sitzungsber. der Wiener Akad.* lxiii. 214.

[2] Külz, *Zeit. Biol.* xxii. 161.

Glycogen is also found in muscles (*see* p. 422), in many fœtal tissues, in white blood-corpuscles, and in numerous invertebrate animals.

Variations in the amount of glycogen in the liver.—In 1843 Cl. Bernard[2] recognised that in the liver was a source of grape-sugar, and that the sugar in the blood did not directly depend on the intake of carbohydrates in the alimentary canal; he found, for instance, that sugar occurred in the blood when the animal was fed on a purely proteid diet. Bernard found sugar in the liver of all animals which were in a healthy condition. The next important step in the investigation of this subject was in the year 1856, when Bernard[3] and Hensen[4] independently of each other prepared from the liver a carbohydrate which like starch formed an opalescent solution with water, and was convertible into sugar by saliva or other diastatic ferments. This is the substance we now know as glycogen. Brücke,[5] Abeles,[6] and Külz[7] have since that time improved on the original methods adopted for its preparation.

Bernard stated that the fœtal liver contains little or no glycogen, and considered that the placenta, which contains glycogen, takes the place of the liver as a source of sugar in intra-uterine life. Hoppe-Seyler,[8] however, finds that the fœtal liver like most other fœtal tissues, including the placenta, contains abundance of glycogen, and in new-born dogs Demant[9] found the glycogen to be more abundant than in adult animals. Salomon[10] has made similar observations on still-born children.

During inanition the glycogen of the liver disappears; none can be found in the livers of rabbits after six to nine days' abstinence from food; other animals have also been investigated with similar results (Luchsinger,[11] Weiss,[12] Külz,[13] Aldehoff,[14] and others). The hepatic glycogen seems to disappear more quickly than the muscle-glycogen. During hibernation the liver continues to be rich in glycogen (Aeby,[15] Voit[16]).

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[1] Cramer, *Ibid.* xxiv. 67. Cramer's careful experiments corrected the statement, originally made by v. Wittich (*Centralbl. med. Wiss.* 1875, No. 8), that different parts of the liver contain different percentages of glycogen.

[2] Bernard, *Nouvelle fonction du foie*, 1853; *Arch. générales de méd.* Oct. 1848.

[3] Bernard, *Gaz. méd. de Paris*, No. 13, 1857; *Compt. rend.* xliv. 578.

[4] Hensen, *Verhandl. d. phys. med. Gesellsch. zu Würzburg*, July 1856, vol. vii. p. 219; *Arch. path. Anat.* xi. 395.

[5] *Loc. cit.*

[6] Abeles, *Wien. med. Jahrbücher*, 1877, p. 551.

[7] *Loc. cit.*

[8] Hoppe-Seyler, *Physiol. Chem.* p. 708.

[9] Demant, *Zeit. physiol. Chem.* xi. 142.

[10] Salomon, *Centralbl. med. Wiss.* 1874, No. 47.

[11] *Pflüger's Archiv*, xviii. 472.

[12] Weiss, *Wien. Akad. Sitzungsber.* lxvii.

[13] Külz, *Sitzungsb. d. Marburger Gesell.* 1876, No. 5.

[14] Aldehoff, *Zeit. Biol.* xxv. 137.

[15] Aeby, *Arch. exp. Path. u. Pharm.* iii. 184.

[16] Voit, *Zeit. Biol.* xii. 269.

A few hours' active exercise causes a greater reduction of the hepatic glycogen than days of starvation (Külz[1]); muscular exercise also reduces the amount of glycogen in muscle (*see* p. 424). Strychnine poisoning has the same result, even when it does not produce convulsions; and, strangely enough, curare, which abolishes muscular movement, has the same effect; in both cases the animals become at the same time diabetic (Demant[2]).

In fever, the liver of human beings is found *post mortem* to be almost free from glycogen and sugar (Bernard). In diabetes the glycogenic function of the liver is deranged, so that an increased quantity of sugar enters the circulation.

In poisoning by arsenic and phosphorus, the glycogen of the liver is diminished. These drugs produce a fatty degeneration of the liver-cells. In other forms of fatty liver the glycogen is also diminished in quantity, or it may be absent.

It has been found experimentally in animals that ligature of the bile-ducts, no doubt by interfering with the normal metabolism in the liver-cells, also causes a diminution of the hepatic glycogen (v. Wittich).

Formation of glycogen in the liver.—Bernard supposed that proteid food was the source of the hepatic glycogen; Pavy, on the other hand, considered that it was from carbohydrate food alone that glycogen was derived; the usual view accepted at the present time is that both varieties of food may act as sources of glycogen. The glycogen is most abundant after carbohydrate food, but it also occurs in the liver of flesh-feeding animals, and in animals kept exclusively on a proteid diet. Glycogen is not formed from fat.

The following are the chief experimental facts bearing on this subject:—

Grape sugar, starch, dextrin, cane sugar, inulin, fruit sugar, milk sugar, and lichenin given as food increase the amount of glycogen in the liver (Dock,[3] Luchsinger,[4] Frerichs,[5] Külz, Bernard, Tscherinoff,[6] Pink,[7] Komanos,[8] Salomon, v. Mering[9]). Gum arabic, inosite, mannite, erythrite, and quercite have no such effect (Salomon, Külz, v. Mering, Luchsinger).

Fats and soaps are also inactive (Bernard, Tscherinoff, McDonnel,[10] Luchsinger, Külz).

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[1] Külz, *Pflüger's Arch.* xxiv.

[2] Demant, *Zeit. physiol. Chem.* x. 442.

[3] Dock, *Pflüger's Archiv*, v. 571.

[4] Luchsinger, *Ibid.* viii. 289.

[5] Frerichs, *Diss.* Würzburg, 1876.

[6] Tscherinoff, *Wien. Akad. Sitzungsb.* li. (2), 412.

[7] Pink, *Diss.* Königsberg, 1874.

[8] Komanos, *Diss.* Strasburg, 1875.

[9] v. Mering, *Pflüger's Archiv*, xiv. 274.

[10] McDonnel, *Compt. rend.* lx. 693.

Gelatin increases the amount of glycogen in the liver (Bernard, Salomon, Luchsinger).

Proteid foods also increase the amount of glycogen in the liver (Bernard, Finn,[1] v. Mering, Naunyn [2]). Some observers have failed to make out any marked increase (Dock, Weiss, Luchsinger).

Glycerin undoubtedly causes an increase of the liver glycogen (Weiss, Luchsinger, Salomon).

Ammonium carbonate increases the glycogen also (Röhmann [3]), and certain amido-compounds (asparagine, glycocine) act similarly (Röhmann [4]). As sodium carbonate does not act in this way, it is suggested that ammonium carbonate does not exert its influence by reason of its alkalinity, but that ammonia and a carbohydrate entering the liver together may form a new compound which will split into glycogen on the one hand, and a nitrogenous product, such as urea, on the other.

Forster [5] obtained an increase in the glycogen of the liver by injecting a concentrated solution of sugar into the portal vein, and at the same time the urea in the urine was increased. Luchsinger [6] found that, by passing a stream of arterial blood containing grape sugar in solution through a liver just removed from the body, glycogen continued to be formed, and Seegen and Kratschmer [7] found that a calf's liver after removal from the body continued to form glycogen even though no blood was passed through it.

Prausnitz,[8] from experiments in feeding hens on cane sugar, concludes that the quantities of glycogen in the whole body, in the liver, and in the muscles run closely parallel to one another; the maximum of glycogen-formation, as evidenced by the quantity found after death, occurs twenty hours after feeding. This is somewhat later than is stated by previous observers, and is certainly not coincident with the maximum of bile-formation.

From such an enumeration of the substances that have been found to cause an increase of the liver glycogen, one would be justified in at once concluding that the building up of glycogen in the liver is by no means a simple process. The following suggestive remarks bearing on this subject are taken from a paper by E. Pflüger [9] on synthetical processes in the animal organism.

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[1] Finn, *Würzburger Verhandl. d. phys. med. Ges.* N.F. xi. No. 192.

[2] Naunyn, *Arch. f. exp. Path. u. Pharm.* iii. 85.

[3] Röhmann, *Centralbl. f. klin. Med.* 1884, No. 35.

[4] Röhmann, *Pflüger's Arch.* xxxix. 21.

[5] *Zeit. Biol.* xi. 515.

[6] *Diss.* Zürich, 1875, p. 62.

[7] *Pflüger's Archiv*, xxii. 88.

[8] *Zeit. Biol.* xxvi. 371.

[9] *Pflüger's Archiv*, xlii. 144.

'A living liver free from glycogen will again form that substance not only from carbohydrates, but from gelatin, proteid, or from glycerin. v. Mering[1] fed dogs on phloridzin, whereby they became diabetic, and in a few days all carbohydrate materials in the body had been discharged as sugar. If now the same drug were given to the same animals after a few days' interval during which they had no food, they once more became intensely diabetic, and the quantity of sugar passed was so enormous that it cannot be supposed to have come from the drug itself. It must therefore have been formed from the proteid substances in the animals' tissues. One explanation of the way in which glycogen is formed after the administration of glycerin is the well-known 'economy theory';[2] another is that glycerin and similar substances act as stimuli to the activity of the liver-cells. It certainly cannot be supposed that glycogen is directly formed from the substance administered, or at least not in all cases; for instance, from ammonium carbonate.

'The question then arises as to the genetic relationship existing between glycogen and albumin. Experiments outside the body on the decomposition products of proteids have in no case yielded a carbohydrate, and not only that, but proteids never yield any of the commoner decomposition products of carbohydrates such as mucic acid, tartaric acid, &c. Still we have the formation of glycogen taking place in the liver, when no food but albuminous food is taken.

'The following general considerations will, however, lead to a better understanding of the subject. The chemical differences between animal and vegetable cells are not so great as was at one time supposed; their chemical composition, so far as it is known, is the same; all living cells breathe oxygen and produce carbonic anhydride, water, and amide compounds. Synthetic processes are more highly developed in chlorophyll-holding plants, but they also occur in animal cells. As instances of synthetic processes occurring in animal cells, the formation of hippuric acid from glycocine and benzoic acid, or of an ethereal sulphate from phenol and sulphuric acid, may be taken. A special kind of synthesis must moreover occur in the retrogressive metamorphoses of proteids that lead to the formation of uric acid and urea. In albumin itself, and in the derivatives of albumin obtained in the laboratory like indole and leucine, the number of carbon atoms is much greater than that of nitrogen atoms, but in many of the products of metamorphoses in the body, the nitrogen and carbon atoms are nearly equal in number, or, as in the cases of urea and guanidine, the nitrogen atoms are the more numerous. The importance of such synthesis occurring in living cells, resulting in the formation of molecules containing cyanogen, has been long insisted on by Pflüger himself (*see* p. 116).

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[1] *Verhandl. VI. Congresses innerer Med.* Wiesbaden, 1887.

[2] This theory may be briefly stated thus: the glycerin is used in combustion instead of glycogen, so allowing the latter body to accumulate.

'Researches on the formation of fat within the body show that here again there are undoubtedly syntheses occurring as the result of the activity of living cells; in fact, here again reactions occur which cannot be repeated in the laboratory or explained by any known chemical law; they are probably therefore the result of a breaking down of molecules in the first place, and the living cells then build up entirely new materials of a complex nature from the simple carbon compounds so liberated. In the synthesis of fats from carbohydrates the group CH.OH must be changed into CH_2, and in the formation of carbohydrate (glycogen) from proteid the group CH_2 must be changed into CH.OH; in both cases numbers of these groups become linked together.

'From considerations such as these it is seen that the formation of a carbohydrate from a proteid is by no means a solitary instance of a chemical reaction occurring in the body which cannot be explained by known chemical laws. The more the chemistry of the living cell is studied, the more is it demonstrated how profound are the chemical revolutions it may bring about in organic compounds, and especially in proteids, which are substances particularly prone to undergo intramolecular changes.'

The formation of glycogen ($C_6H_{10}O_5$) from sugar ($C_6H_{10}O_6$) is comparatively a simple process, consisting of the removal of a molecule of water. The 'economy theory' of the formation of glycogen was advanced by Tscherinoff as an attempt to explain the increase in the liver of that substance which followed on the administration of substances such as glycerin out of which the chemist in the laboratory was unable to make glycogen. The glycerin or other substance given was supposed, by itself undergoing combustion, to spare the glycogen which would have otherwise been used for the purpose, and so lead to an accumulation of the glycogen. There are, however, two weak points which render the economy theory a very unsatisfactory one; one is that the administration of other eminently combustible substances like lactic acid or alcohol do not lead to a similar accumulation of the hepatic glycogen; and the other weak point is this, that the theory cannot be applied universally; if it be extended so as to have a universal application, it comes to the same thing as saying that the glycogen is always the result of the proteid metabolism of the cells. Many careful experiments have, however, shown that glycogen is directly formed from carbohydrate food. I will here quote one such experiment from a paper recently published by E. Voit.[1] The experiment was made on a goose, which after a period of 4½ days' inanition was fed for the following five days on 766·2 grains of dry rice. The animal was then killed and the glycogen estimated by Brücke's method. The results of the analysis are given in tabular form below.

Supposing that the glycogen had entirely disappeared during the period of inanition, there was thus a formation of at least 44·17 grammes during the subsequent five days ; this does not take into account any small quantities that might have been present in the skin and fatty tissue, nor the quantity used by the organism during the five days before death. From analyses of the urine and fæces during this time it was found that 8·2 grammes of nitrogen were excreted, of which 4·7 only could have been derived from the rice given as food. For every gramme of nitrogen in the proteid 1·17 gramme of carbon would be available for the formation of glycogen ; 8·2 grammes of nitrogen would therefore correspond to 13·94 grammes of carbon, and that would account for 33·7 grammes of glycogen. The remaining 10·4 grammes must therefore have been formed from the carbohydrate of the food.

Organ	Weight	Glycogen	
		Total amount	Percentage
Liver	205·5 gr.	21·6 gr.	10·5
Muscles	1327·5 ,,	17·52 ,,	1·32
Other tissues (except skin & fat)	382·5 ,,	5·05 ,,	—

The fate of the liver glycogen.—In the preceding section we have attempted to give an answer to the difficult question, From what is glycogen formed ? We have now to face the equally hard problem, What becomes of the glycogen ?

The original teaching of Bernard was that in the normal condition of the liver a certain amount of the glycogen is converted into sugar, and that this is carried from the liver by the hepatic vein, and thence to the other organs and tissues of the body, which require it for their nutrition. Although this theory has passed through many vicissitudes, Bernard's teaching is practically the same as that now accepted. Renewed research has shown that many of the objections which have been raised to it are groundless.

After Bernard the question was taken up by Pavy,[2] who stated that if the liver be removed from a healthy animal with sufficient rapidity after death, and boiled or scalded so as to kill a supposed ferment, no sugar was obtainable from it, and that during life the blood in the hepatic vein contained no more sugar that the portal blood. Pavy,[1] however, subsequently admitted that the normal liver contains 0·2 to 0·6 part per 1000 of sugar. The large quantity of sugar found in a liver after death was attributed to the action of a ferment which was considered to be formed in the blood from the solution of the blood-corpuscles.[2] This ferment was considered to be a diastatic ferment, i.e. one which like ptyalin and diastase converts starch and glycogen into sugar.

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[1] E. Voit, *Zeit. Biol.* xxv. 543.

[2] Pavy, *Guy's Hosp. Rep.* 1858, vol. iv. p. 291; *Proc. Roy. Soc.* ix. 300; *Researches on the Nature and Treatment of Diabetes*, London, 1862.

The quantity of sugar in the hepatic vein as compared with that in the portal vein is a very important point to settle in connection with this question, and this apparently simple investigation has been the subject of very contradictory statements. v. Mering[3] found less sugar in the blood of the hepatic than in that of the portal vein; Bleile,[4] like Bernard, found more; and Abeles,[5] like Pavy, found about the same amount in the two varieties of blood. Prof. M. Foster, speaking on the subject in his 'Text Book of Physiology,'[6] says: 'In view of this conflicting evidence we shall not go far wrong in assuming that Bernard's view is not as yet clearly disproved. The quantitative determination of sugar in the blood is open to many sources of error. When the quantity of blood which is continually flowing through the liver is taken into consideration it is obvious that an amount of sugar which in the specimen of blood taken for examination fell within the limits of errors of observation might, when multiplied by the whole quantity of blood and by the number of times it passed through the liver in a certain time, reach dimensions quite sufficient to account for the conversion into sugar of the whole of the glycogen present in the liver at a given time.'

Others, puzzled by Pavy's researches on the very slight increase or absence of any increase of sugar in the hepatic blood, considered that the glycogen of the liver is converted, not into sugar at all, but into fat. This view was chiefly based on the fact that carbohydrate food may be in some way or other a source of the fat of the body. In view of recent researches, however, this theory of the fate of the liver glycogen is unnecessary.

Among recent investigators, Seegen has done most to add to our knowledge on the subject. Many of his conclusions have not, however, met with general acceptance. It will be convenient to discuss these researches, not in chronological order, but under the following heads:—

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[1] *Croonian Lectures on Diabetes*, 1878.

[2] Tiegel, *Pflüger's Archiv*, vi. 249.

[3] v. Mering, *Arch. f. Anat. u. Physiol.* 1877; *Physiol. Abth.* p. 412.

[4] Bleile, *Ibid.* 1879, p. 75.

[5] Abeles, *Wien. med. Jahrbücher*, 1875, vol. iii.

[6] Fifth edition, p. 726.

The percentage of sugar in different kinds of blood.—From a large number of experiments Seegen[1] gives the following averages:—

Normal amount of sugar in cardiac and arterial blood		0·1 – 0·15 p.c.
„ „	portal blood	0·119 „
„ „	hepatic blood	0·23 „

The following four experiments[2] illustrate the same fact, viz. that hepatic blood contains, roughly speaking, twice as much sugar as portal blood:—

	Percentage of sugar	
	Portal blood	Hepatic blood
1.	0·101	0·258
2.	0·090	0·175
3.	0·107	0·209
4.	0·120	0·287

What kind of sugar is present in the blood.—Although the sugar formed from glycogen by diastatic ferments is maltose, that found in the blood leaving the liver is dextrose (Külz, Seegen,[3] Eves[4]). Chittenden and Lambert[5] speak of the sugar as a mixture of maltose and dextrose, but Seegen has shown that this is probably due to the fact of small quantities of dextrin and cane-sugar, which are partly absorbed as such from the alimentary canal, having been reckoned as maltose.

Formation of sugar in excised livers.—The liver continues to form sugar after death, and according to Seegen[6] the glycogen does not diminish in a corresponding ratio. He concludes, therefore, that the glycogen does not furnish the sugar, but fulfils some other, at present unknown, function. If pieces of excised liver be placed in contact with solution of peptone, sugar is produced, and the same occurs with fat. He therefore concludes that the liver forms its sugar from proteid and from fat. This conclusion is, as Hoppe-Seyler[7] states, not to be accepted without fuller inquiry. Seegen does not show, for instance, that the peptone diminishes in proportion as the sugar increases. An equally possible explanation is that the peptone merely stimulates the activity of whatever agent it is that produces sugar in the liver after death; or a third possible explanation—and a very probable one, as it was on carnivorous animals (dogs) upon which most of the experiments were performed—is that glycogen may be an intermediate product in the formation of sugar from proteids. The figures obtained by other analysts are entirely contrary to Seegen's on this point. Thus Chittenden and Lambert, Böhm and Hoffmann,[1] H. Girard,[2] Abeles[3] and Panornow,[4] and A. Dastre[5] have all arrived at the same conclusion, viz. that as the sugar increases, the glycogen diminishes. I quote the following table from H. Girard's analyses, which brings out this point very well:—

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[1] *Bied. Centralbl.* 1884, p. 747.

[2] Seegen, *Pflüger's Archiv*, xli. 526.

[3] *Ibid.* xl. 48.

[4] *Journ. of Physiol.* v. 350.

[5] *Studies from Lab. Physiol. Chem. Yale Univ.* 1885.

[6] Seegen and Kratschmer, *Pflüger's Archiv*, xxii. 3; xxxvii. 348; xxxix. 121.

[7] *Physiol. Chem.* p. 717.

Animal	10 minutes after death		24 hours after death		48 hours after death	
	Sugar	Glycogen	Sugar	Glycogen	Sugar	Glycogen
Dog 1	0·55	2·12	1·80	0·76	1·75	0·75
„ 2	0·74	4·05	3·00	1·50	3·12	1·38
Cat 1	0·48	5·88	2·95	3·20	3·06	2·88
„ 2	0·62	4·96	3·15	2·08	3·48	1·87
Rabbit 1	0·75	9·56	3·58	6·35	3·85	4·28
„ 2	0·65	10·25	4·12	6·24	4·20	5·05

The ferment theory of the change of glycogen into sugar.—These changes just described that occur after death are no doubt indications of what is always occurring during life; in the normal process of metabolism, glycogen is formed from something else on the one hand, and given out as sugar on the other by the same cells. After death, as the liver-cells retain a certain amount of vitality, the process still continues.[6] There is no necessity to assume the action of any special ferment developed after death to account for the phenomenon observed, and in fact Seegen and Eves have both shown that no such ferment can be extracted from the liver in larger quantity than from any other tissue of the body. We have, for instance, already seen that such a ferment can be obtained from muscle (p. 412), and it seems that diastatic activity is present in all living proteids. The diastatic ferment which is obtainable is, however, not derived from the blood, but from the liver-cells (v. Wittich[1]), and it moreover does not convert glycogen into dextrose, but into maltose.

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[1] *Pflüger's Archiv*, xxiii. 205.

[2] *Ibid.* xli. 294.

[3] *Wiener med. Jahrb.* 1887, p. 383.

[4] Polish paper; Abstract in *Maly's Jahresb.* xvii. 304.

[5] *Arch. de Physiol.* (4), i. 69.

[6] In favour of this view it may be stated that the greatest formation of sugar that occurs in the excised liver takes place within an hour or two after its removal. That the liver retains its vitality for this length of time is supported by the fact that if a stream of blood be passed through an excised liver, it continues to form bile for a couple of hours (Schmulewitsch, *Ber. d. Sächs. Akad. d. Wiss.* 1868)

In a recent research on the influence of glycerin on the liver, W. B. Ransom[2] finds that the administration of glycerin to rabbits prevents the glycosuria that usually follows injury to a certain spot of the medulla oblongata; and also that it prevents the post-mortem change of glycogen into sugar; he therefore concludes that the glycerin checks the glycosuria by inhibiting the formation of sugar in the liver-cells, and that in this way the accumulation of glycogen in the liver is fully explained.

Fat in the liver-cells.—The normal liver contains about 2 to 3 per cent. of fat; in acute yellow atrophy the percentage may rise to 7·6, and in fatty degeneration to 19·5. Fatty degeneration occurs in many wasting diseases, such as phthisis, as a result of chronic alcoholic poisoning, and also as a result of poisoning by phosphorus, arsenic, and antimony.[3] Grohe and Mosler[4] state that in the duchy of Brunswick the peasants give to the geese when producing the famous fatty livers a small quantity of white oxide of antimony every day.

The fat in the liver-cells can be readily seen by the microscope in the form of minute globules. These are especially abundant after a meal, particularly after a fatty meal. These globules are seen in greatest number in the so-called portal zone of a hepatic lobule; that is, in the outer region of the lobule, which is the part to which the portal capillaries are first distributed. In fatty degeneration it is generally noticeable that the morbid process commences in the same region.

The amounts of glycogen and fat in the healthy liver run parallel with one another. In hunger both disappear, on feeding they reappear; and just as glycogen may under certain circumstances be formed from substances other than carbohydrates, so fats seem also to be formed from substances like proteids other than fats. Fat like glycogen is here a result of the metabolic activity of the liver protoplasm.

Other organic constituents of the liver-cells.—Urea, uric acid (especially in birds), xanthine, and hypoxanthine are found in the liver.[5] These substances are instances of the destructive metabolism of proteids; the liver is considered generally to be the organ where a very large amount of the organic bodies of the urine are formed. These pass into the blood-stream and leave the body by the kidney secretion.

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[1] *Pflüger's Arch.* vii. 28.

[2] *Journ. of Physiol.* viii. 99.

[3] A recent paper on the relation of fat in the liver to various toxic agents will be found by Chittenden and Blake, *Studies from Lab. Physiol. Chem. Yale Univ.* iii. 106. *See* also Salkowski, *Virchow's Archiv*, xxxiv. 78.

[4] See Wood's *Therapeutics*, p. 161.

[5] Scherer, *Ann. Chem. Pharm.* cvii. 314; Cloëtta, *Ibid.* xcvii. 289.

When this function of the liver-cells is considered in addition to those already enumerated, we see the vast importance in analytical and especially in synthetical processes that is possessed by the mass of protoplasm we call the liver.

Leucine and tyrosine do not normally occur. They are, however, found in the liver of cases of acute yellow atrophy, and in cases of phosphorus poisoning.[1]

Various other substances have been described by various observers, but do not appear to be constantly present; such as guanine, inosite, scyllite,[2] cystin (in a pathological case),[3] sarco-lactic acid (probably formed after death).[4]

A substance, *jecorin*, containing phosphorus ($C_{105}H_{186}N_5SP_3O_{46}$) has recently been separated from the liver by Drechsel.[5] In its properties it somewhat resembles lecithin; it however like sugar, but unlike lecithin, reduces Fehling's solution. According to Baldi,[6] it occurs also in many other organs—spleen, muscle, brain, &c.

The so-called waxy or amyloid substance replaces the protoplasm of the liver-cells in the condition known as waxy degeneration. In this condition large quantities of cholesterin are also sometimes found in the liver.[3]

The inorganic constituents of the liver.—These have been already enumerated; it is now necessary to add a few words respecting one of the most interesting of these, viz. iron.

Iron in the liver.—This has been the subject of a special research by S. S. Zaleski.[7] The liver was first thoroughly freed from blood by a stream of 2·5 per cent. cane-sugar solution. The quantity of iron in the blood-free liver was found to vary between wide limits, but it was constantly found in organic combinations in the liver-cells, especially with nuclein; and one of the iron-nuclein compounds named *hepatin* was isolated. The iron in these compounds is present in two, probably in three states of oxidation, in the ferrous, ferric, and ferroso-ferric states. Of all the macro-chemical reactions for detecting iron the most delicate were found to be (1) that with potassium thiocyanate and hydrochloric acid, (2) that with potassium ferro- or ferri-cyanide and hydrochloric acid. The green or blue colour produced by the latter test was found to be best adapted for microscopical investigation. After the administration of iron compounds the metal collects in the liver, and Zaleski[1] concludes that iron is excreted by the liver, not by the intestinal glands as other heavy metals are. There can, however, be no doubt that the iron-containing pigment present normally in liver-cells is derived from the blood-corpuscles.

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[1] Sotnitschewsky, *Zeit. physiol. Chem.* iii. 391.

[2] Frerichs and Städeler, *Mitth. d. Züricher naturf. Gesellsch.* 1855.

[3] Hoppe-Seyler, *Physiol. Chemie*, p. 718.

[4] Minkowski (*Arch. exp. Path. u. Pharm.* xxi. 14); Marcuse (*Pflüger's Archiv*, xxxix. 425) and Nebelthau (*Zeit. Biol.* xxv. 123) found that after extirpation of the liver lactic acid appeared in the urine.

[5] *Journ. prakt. Chem.* xxxiii. 425.

[6] *Du Bois Reymond's Archiv*, supplement, 1887, p. 100.

[7] *Zeit. physiol. Chem.* x. 453; xiv. 274. This subject was first brought into prominence by the researches of Quincke (*Deutsch. Arch. f. klin. Med.* xxv. 567; xxvii. 202 xxxiii. 23). *See* also Peters (*Ibid.* xxxii. 182).

The great increase of the iron in the liver in cases of pernicious anæmia has been already alluded to (*see* p. 301); a certain amount of blood is always being destroyed in the liver in health, and this is increased in the disease just mentioned (Hunter,[2] Mott,[3] Delépine[4]).

Dr. Mott has recently recorded three cases which confirm this view of the pathology of the disease. The portal zones of the lobules were so crowded with albumino-ferruginous compounds that the sections when stained with potassium ferrocyanide and hydrochloric acid appeared as if injected. The urine as in most cases (Fagge) was highly coloured. Mott's view of the decomposition that occurs is that the hæmoglobin is acted on by the liver-cells to form urobilin or an allied pigment that appears in the urine, and the iron is left behind and accumulates as the disease progresses. In two of the cases the amount of iron found in the liver by analysis was very large, while that in the spleen was not greater than normal. In the third case the iron was increased in both organs. Hunter[5] has also recorded a similar case, except that he found pathological urobilin instead of normal urobilin in the urine.

Bemmelen[6] from his own researches and from those of Stahel[7] and Graanboom[8] concludes that the normal percentage of iron in the liver is 0·1. In a case of leucæmia this was reduced to 0·01. It is present in greater proportions in the liver of new-born animals, and probably acts as a storehouse of iron subsequently used in the formation of blood-corpuscles (Bunge). Delépine believes that this function persists throughout life.

Certain pathological conditions of the liver.—It has been necessary to allude to several pathological conditions like fatty degeneration, diabetes, &c. in the foregoing paragraphs. I have here merely to add a table which collects together various quantitative analyses that have

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[1] *Chem. Centralbl.* 1888, p. 759.

[2] *Lancet*, ii. 1888, pp. 555, 608, 654. Full references to previous workers will be found in these papers.

[3] *Lancet*, vol. i. 1889, p. 520; vol. i. 1890, p. 287; *Practitioner*, Aug. 1890.

[4] *Practitioner*, August 1890.

[5] *Ibid.* September 1889.

[6] *Zeit. physiol. Chem.* vii. 497.

[7] *Maly's Jahresb.* xi. 423.

[8] *Ibid.* p. 429. *See* also Lapique, *Compt. rend. Soc. Biol.* 1889.

—	v. Bibra		Folwarczny		Frerichs		Oidtmann
	Fatty liver (tubercle)	Typhoid fever	Diabetes	Embolism of hepatic artery	Fatty liver	Cirrhotic liver	Syphilitic liver in new-born child
Water	71·0	75·1	75·3	80·7	73·0	80·2	82·5
Soluble proteids	1·3	2·6	6·7	2·1	3·6	3·5	16·5
Gelatin	4·4	4·0	1·1	1·1	1·9	11·5	
Extractives	2·6	4·5	2·2	3·6			
Fat	17·4	3·3	1·9	2·4	17·2	2·2	
Insoluble tissues	3·1	10·2	11·7	8·9	4·0	3·6	9·1
Salts	—		0·9	0·9	—	—	

been made, and for which I am indebted to Charles' 'Physiological Chemistry.'[1] This table is chiefly interesting as showing the great increase of fat in fatty livers, and of gelatin due to the overgrowth of connective tissue in a cirrhotic liver. In a liver that had undergone acute atrophy Röhmann[2] found albumose and peptone, sarco-lactic acid and a mixture of amido-acids, alanine, leucine, and tyrosine being the most abundant. The latter were absent from the urine, which contained, however, excess of aromatic oxy-acids.

THE SPLEEN

'The spleen is invested with a fibrous and muscular *capsule*, and this again has a covering derived from the *serous* membrane. The capsule sends fibrous bands or *trabeculæ* into the organ, and these join with similar trabeculæ which pass in at the hilus with the blood-vessels. In the interstices of the framework so formed lies a *pulpy substance* containing blood, and therefore of a red colour, within which are seen small whitish specks, the *Malpighian corpuscles*. These are composed of lymphoid tissue which is gathered into masses which envelop the smaller arteries, while the pulp which everywhere surrounds them is composed of a close network of flattened and branched cells like connective-tissue corpuscles. Coursing through the pulp and communicating with its interstices are capillaries connected with the terminations of the arteries: in other parts venous channels arise from the pulp and bring the blood which has passed into its interstices from the arterial capillaries towards the larger veins of the organ which run in the trabeculæ and are by them conducted to the hilus.'

'The cellular elements of the pulp are of three kinds, viz. peculiar large amœboid cells called *splenic cells*, lymph-corpuscles, and the branched cells which form the sponge-work. The first named are frequently found to contain coloured blood-corpuscles in their interior in various stages of transformation into pigment.'

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[1] P. 355.

[2] *Berlin. klin. Woch.* 1888, Nos. 43 and 44.

The foregoing brief account of the histology of the spleen taken *verbatim* from Schäfer's 'Essentials'[1] shows us the number of microscopic elements with which we have to deal, and thus the large number of chemical substances obtainable from the spleen is fully accounted for.

Chemical composition of the spleen.—Oidtmann[2] states that the percentage of water in the adult human spleen varies from 69·4 to 77·5; the solids from 31·6 to 22·5, of which from 30·1 to 21·6 consist of organic, and from 1·1 to 0·9 of inorganic matters.

The organic constituents that have been described are proteids and hæmoglobin; xanthine,[3] hypoxanthine,[3] uric acid,[4] glycogen,[5] inosite,[6] scyllite,[7] cerebrin,[8] cholesterin,[8] lecithin,[8] and jecorin[9] in small quantities. Gelatin and mucin are also present and are derived from the supporting connective tissue.

Various fatty acids (formic, acetic, butyric) described by Scherer[10] are no doubt derived during the process of distillation from the proteids and hæmoglobin (Hoppe-Seyler).

Leucine and tyrosine, which are often found, are the result of putrefactive changes; they are absent in the fresh organ (Hoppe-Seyler).

Lactic and succinic acids were found by Gorup-Besanez. The variety of lactic acid present is sarco-lactic acid (Hirschler[11]). This appears to be especially formed after death, giving to the spleen an acid reaction. During life the spleen is alkaline.

The inorganic constituents.—These are very much like those found in the liver. Oidtmann gives the following analysis; the numbers are percentages of the ash:—Soda, 35–45; potash, 9–17; lime, 7; phosphoric acid, 18–30; oxide of iron, 7–16; chlorine, 0·5–1·3; sulphuric acid, 1·5–2·5; silica, 0·2–0·7; manganese, copper, and lead in traces.

One of the most interesting of these constituents is iron. In the splenic pulp of old horses H. Nasse[12] found that nearly 5 per cent. of the dry residue consisted of iron. The iron is present in organic combinations, and mostly as hæmoglobin. Hoppe-Seyler regards other organic compounds containing iron which have been described as artificial or post-mortem decomposition products of hæmoglobin. Lapique states that the spleen of young animals contains less iron than that of adults, which is the opposite to what is the case in the liver.

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[1] P. 159.

[2] *Loc. cit.*

[3] Scherer, *Ann. Chem. Pharm.* cvii. 314; Städeler, *Ibid.* cxvi. 102; Neubauer, *Zeit. anal. Chem.* vi. 33; Gorup-Besanez, *Ann. Chem. Pharm.* xcviii. 1; Cloëtta, *Ibid.* xcix. 289.

[4] Scherer, Cloëtta, Gorup-Besanez.

[5] Hoppe-Seyler, *Med. Chem. Unters.* iv. 495; Abeles, *Centralbl. med. Wiss.* 1878, No. 5.

[6] Cloëtta, Scherer.

[7] Frerichs and Städeler, *Mitth. Züricher naturf. Gesell.* 1855.

[8] Hoppe-Seyler.

[9] Baldi, *Du Bois Reymond's Arch.* supp. 1887, p. 100.

[10] *Verhandl. Würzburger phys. med. Gesell.* ii. 323.

[11] *Zeit. physiol. Chem.* xi. 41.

[12] Quoted by Hoppe-Seyler, *Physiol. Chem.* p. 7[illegible].

Functions of the spleen.—The lymphoid tissue is no doubt a place for the manufacture of white blood-corpuscles. With regard to the red corpuscles, some hold that they are destroyed, others that they are formed, and others again that both processes may occur in the spleen.

The splenic cells are also believed to liberate hæmoglobin from 'effete' corpuscles, which, passing to the liver, is there transformed into bile-pigment. This is erroneous, but the question will be considered again in connection with the bile. Schiff and Herzen[1] supposed that the spleen also manufactures the pancreatic ferment. Mosler has shown that this is, however, probably not the case.

The spleen has been removed from healthy animals (Galen) and also from the human subject without any bad results following. In certain animals, e.g. the dog, the operation has been followed by hypertrophy of other hæmopoietic tissues (lymphatic glands and red marrow) ; but in certain other animals, e.g. the rabbit, this does not appear to be the case.[2] In the disease known as splenic leucocythæmia, in which the spleen is hypertrophied, there is a great increase of the white corpuscles of the blood (*see* p. 302). In this disease Charcot's crystals (*see* p. 303) are also found in the splenic pulp.

In progressive pernicious anæmia the destruction of blood in the liver and also in the spleen is much increased, and so an increased quantity of iron is found in those organs (*see* pp. 301, 552).

The administration of toluylenediamine produces similar results to those observed in pernicious anæmia (Engel and Kiener,[3] Hunter).

In attacks of ague the spleen becomes enlarged, and this is apparently connected with increase of uric acid in the urine. After many attacks the spleen becomes permanently enlarged and hard from the overgrowth of connective tissue.

LYMPHATIC GLANDS

These structures are composed of lymphoid tissue with an investing capsule and trabeculæ of fibrous tissue.

The connective-tissue structures yield the same chemical materials as this tissue does in general, especially gelatin and mucin. The lymph-cells are simply white blood-corpuscles, the chemistry of which has been already described (p. 258).

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[1] Virchow, Hirsch, *Med. Jahresb.* 1870, i. 100 (original paper in Italian). *See* also A. Herzen, *Pflüger's Archiv*, xxx. 295 and 308.

[2] Tizzoni, *Internat. Monatsschrift für Anat. und Physiol.* ix. 143.

[3] *Compt. rend.* cv. 465.

In a lymphatic gland about two-thirds are water, the remainder solids.

The gland is alkaline during life, and turns acid after death. The acid present is sarco-lactic acid (Hirschler[1]). In the overgrowth of lymphoid tissue that occurs in scrofula and tubercle, there is a great tendency for the new tissue to undergo degenerative changes, caseation and softening, leading to the formation of cavities and abscesses. In the condition of hypertrophy known as lymphadenoma, this tendency is absent.

THYMUS

This body is also lymphoid tissue, and contains the same substances as the lymphatic glands.

Its cells, like those of the lymphatic glands, have been already described in connection with the blood (p. 258).

The so-called 'concentric corpuscles,' which are peculiar to the thymus, do not seem to yield any special chemical substance.

Towards puberty the thymus undergoes fatty degeneration, and is a mere mass of adipose tissue in the adult.

The presence of extractives like xanthine, hypoxanthine, &c. has been noted by Scherer, Gorup-Besanez, Frerichs, Städeler, &c. whose writings have already been referred to. In fact these substances appear to be constantly present in all structures rich in cellular elements.

Schindler[2] has estimated these nitrogenous bases quantitatively in the thymus of the calf, with the following results :—

Percentage in	Adenine	Hypoxanthine	Guanine	Xanthine
Fresh tissue . . .	0·179	0·0023	0·0075	0·038
Dry tissue	1·919	0·218	0·071	0·360

The high percentage of adenine (a base derived from nuclein; see p. 203) is especially noteworthy.

Like all the other organs also that we have examined, the reaction, alkaline during life, becomes rapidly acid after death. This acid is sarco-lactic acid (Moscatelli[3]).

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[1] *Zeit. physiol. Chem.* xi. 41.

[2] Schindler, *Zeit. physiol. Chem.* xiii. 438.

[3] *Zeit. physiol. Chem.* xii. 416.

THYROID

This is also a cellular organ, and proteids (including globulin and a mucin-like substance) and various extractives have been found in it (fatty acids, xanthine, hypoxanthine, &c. by Gorup-Besanez, Scherer, Frerichs, and Städeler). Alkaline in life, it becomes acid after death: this is due to sarco-lactic acid (Moscatelli).

In the adult, the mucin-like material of the alveoli is converted into colloid substance, the properties of which were described in connection with ovarian tumours (p. 353).

Cysts of the thyroid.—In the simple large cysts of the thyroid the fluid is richly albuminous, containing 7 to 8 per cent. of proteids consisting of both serum-globulin and serum-albumin. They may, however, be sometimes filled with colloid material, and very often numerous crystals of cholesterin are seen in the liquid.

Altered blood-corpuscles and altered blood-pigment, such as methæmoglobin or crystals of hæmatoidin, are often found (Hoppe-Seyler [1]).

Myxœdema.—The most constant pathological condition in this disease is atrophy of the thyroid gland, its proper substance being replaced by fibrous tissue. The condition of the blood and connective tissues in this disease has been already fully described (pp. 304 and 501). Horsley believes that some of the degenerative changes of old age may also be attributed to wasting of the thyroid. It is, however, possible that in this case the wasting of the thyroid is not the cause of senile decay, but only a part of the general wasting that occurs in old age.

SUPRARENAL BODY

The function of this organ is unknown, like that of so many of the other so-called ductless glands. It is composed again largely of cells, and in addition to the usual extractives present others have been found by different observers; thus Cloëz and Vulpian [2] found hippuric and taurocholic acids, Seligsohn [3] found benzoic acid and taurine; Holm [4] also found taurine. It is possible that these substances may be absorbed from the neighbouring gall-bladder and kidney. Külz [5] found inosite to be present.

The medulla of the suprarenal is rich in nervous elements, and contains a substance which is soluble in water, and which furnishes a red pigment on exposure to the sunlight. This substance is differently coloured by various reagents, among which may be mentioned ferric chloride, which stains it green or blue.

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[1] *Physiol. Chem.* p. 722.

[2] *Compt. rend.* ii. 1857, p. 10; *Gaz. méd. de Paris*, 1858, No. 24.

[3] *Diss.* Berlin, 1858.

[4] *Journ. prakt. Chem.* c. 150.

[5] *Sitz. Marburger Ges. zu Beford. d. ges. Naturwiss*, 1876, No. 4.

Hæmochromogen has been described in the medulla by MacMunn; the bearing of this observation on the relation of the gland to Addison's disease has, however, been discussed already (p. 304).

With regard to the inorganic constituents, the high percentage of potassium phosphate is no doubt due to the large amount of nervous matter present.

An aqueous extract of the suprarenals is highly poisonous (Foa and Pellacani, 1883): this is due to the presence of the alkaloid neurine (Marino-Zuco[1]); that is what one would expect from the large amount of nervous matter present.

PANCREAS

This organ is alkaline in reaction during life, and rapidly becomes acid after death.

The solids are like those usually obtained from cellular organs; viz. proteids, extractives (guanine,[2] xanthine,[2] hypoxanthine,[2] leucine,[3] tyrosine,[3] uric acid, lactic acid, inosite), and a small proportion of inorganic salts.

An extract of pancreas is an active digestive fluid, and has the same action as pancreatic juice. This subject will be considered fully in connection with digestion.

Extirpation of the pancreas causes glycosuria (*see* p. 663).

SALIVARY GLANDS

The cells of the submaxillary gland contain proteids of which the most abundant is a nucleo-albumin; they also contain mucin, which passes into the saliva (Hammarsten[4]). The sublingual is similar. The parotid cells contain no mucin. A small amount of mucin is, however, obtainable from the investing connective tissue. In myxœdema (p. 504) the parotid cells, however, undergo mucoid degeneration. An extract of the salivary glands exerts a similar diastatic power to that of saliva, as it contains ptyalin. The action of saliva will be fully considered in connection with digestion.

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[1] *Gazzetta*, xviii. 199; *Chem. Soc. Journal*, Abst. 1889, p. 290.

[2] Scherer, *Ann. Chem. Pharm.* cxii. 276.

[3] Virchow, Frerichs, and Städeler, Hoppe-Seyler, *Physiol. Chem.* p. 260. These substances are present in the perfectly fresh organ, and are not the result of putrefaction, as in the spleen.

[4] *Zeit. physiol. Chem.* xii. 163.

KIDNEYS

This is another organ, rich in cells, with a supporting framework of connective tissue, and one has for the most part merely to repeat what has been already said for such organs.

The cells are arranged to line large numbers of tubules; the relation of these to the blood-vessels, and other facts interesting from the point of view of secretion, will be dealt with in our consideration of the urine. Here we have merely to deal with the general chemical composition of the organ itself.

During life the reaction of the renal tissue is alkaline, after death it becomes rapidly acid. Its specific gravity averages 1050.

Gottwalt[1] gives the following numbers relating to the amount of proteids[2] and albuminoids, obtainable from kidneys freed from blood; six analyses were made :—

	Per cent.
Albumin	1·116–1·394
Globulins	8·633–9·225
Other proteids	1·436–1·598
Gelatin	0·996–1·849
Mucin	traces

The following extractives have been obtained by various observers: Xanthine, hypoxanthine, creatine, taurine, leucine, cystin, urea, uric acid, glycogen, and inosite.

The kidney also contains a small proportion of inorganic salts.

Pathological conditions.—In cases of poisoning by alcohol or phosphorus, in septicæmia, in various specific fevers, and in certain forms of Bright's disease, the renal cells undergo a fatty degeneration: this in its early stages produces what is called cloudy swelling.

In certain other forms of Bright's disease, the interstitial connective tissue may be more particularly affected, leading to its overgrowth. In this condition the amount of gelatin and mucin obtainable from the kidney is increased.

In gout, there is not only a hardened kidney, due to connective-tissue overgrowth, but deposits of urate of soda may also occur, both within and around the tubules (*see* p. 510).

The kidney may, like the liver and spleen, undergo waxy degeneration. The following is an analysis of such a kidney by Lambling :—[1]

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[1] Gottwalt, *Zeit. physiol. Chem.* iv. 431.

[2] I have found that the proteids present in the kidney are globulins, one of which coagulates at about 50° C., and the other at 70°–75°; there is also a nucleo-albumin which becomes viscous on mixing it with solutions of sodium chloride, as is the case with lymph-cells (*see* p. 260). *See Proc. Physiol. Soc.* 1890, p. vii.

	Per cent.
Albumin	0·792
Globulins	5·553
Other proteids	0·485
Gelatin	2·685
Waxy substance	0·992

The method of analysis was the same as that adopted by Gottwalt. The waxy substance was isolated by the method of Friedreich and Kekule,[2] and then purified by Kühne's[3] method by artificial digestion with gastric juice and subsequent treatment with baryta-water. There is a diminution in the percentage of proteids as compared with healthy kidneys ; but the amount of waxy substance seems small considering the advanced state of degeneration revealed by the microscope. Lambling considers it possible that the swollen appearance may be in part due to the formation of a substance of the nature of the hyalins described by Krukenberg (*see* p. 486).

Many other morbid conditions, such as abscesses, new growths, &c., may attack the kidney, but have no special chemical interest. The contents of cysts in the kidney have been already described (p. 353).

LUNGS

The lung is composed of many tissues, and thus its chemical constituents are also very numerous. The tissues present are epithelium, connective tissue, elastic tissue, cartilage, and involuntary muscle.

The constituents of the lung are, therefore, proteids, gelatin, mucin, elastin, chondrin. The extractives obtainable are lecithin, leucine, taurine (in oxen), uric acid, inosite. The embryonic lung is rich in glycogen ; in the lung of the embryo sheep, it has been found in as large an amount as 50 per cent. of the dry solids ; it is absent from the adult lung. Lastly there is a small percentage of inorganic salts, chiefly alkaline phosphates and sodium chloride. Small quantities of sulphates and of calcium, magnesium, silica, and iron, are also found.

Pathological conditions.—*The black pigment* present in the lungs of dwellers in smoky atmospheres consists principally of carbon.

Calcareous concretions may occur in the lungs and other parts of the respiratory tract. They have the same composition as similar concretions elsewhere, consisting of lime salts (especially the phosphate and carbonate) mixed with small quantities of organic substances, like mucin and albumin.

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[1] *Compt. rend. Soc. biol.* (2), v. 51.

[2] *Virchow's Archiv*, xvi. 58.

[3] *Maly's Jahresb.* iii. 31.

In tubercle and phthisis generally, the chemical composition of the lung differs with the very various physical conditions that may be present, such as consolidation, fibroid overgrowth, softening, breaking down, calcification of tubercular deposit, &c. The term *caseation* as applied to a certain stage in the breaking down of a tubercle (which in origin is a mass of lymphoid tissue) is one derived from the cheesy appearance of the deposit; there is no proof that any substance of the nature of casein is formed. It appears to be a stage in the fatty degeneration of the cells.

The presence of the tubercle bacillus in cases of phthisis is constant. The very remarkable statement has been made by E. Freund,[1] that the tissues, blood, and pus of tuberculous patients contain cellulose, and apparently the amount of cellulose stated to be present is greater than would be accounted for by the presence of cellulose in the cell-walls of the bacilli themselves.

In pneumonia, the alveoli become filled with proliferated cells, and lymph exuded from the blood-vessels; the lymph coagulates, and thus the lung tissue is solidified, producing the condition known as *hepatisation.* Budde[2] attributes the coagulation that occurs to the presence of a large excess of very active fibrin-ferment in the tissues in this disease. When speaking of the occurrence of intravascular coagulation (p. 305), the fact was mentioned that solution of the clot often takes place with great rapidity; a vessel that is hard to the touch like whip-cord, from the presence of a clot within it, may in a few hours become perfectly pervious. The same holds with regard to the lung in pneumonia. Every clinical observer is familar with the rapid re-solution that occurs in cases of recovery from pneumonia.

The functions of the lung in respiration (Chap. XIX), and the composition and variations in the sputa in different conditions (p. 447), have been already considered. (For Charcot's Crystals *see* pp. 303, 563.)

TESTIS

The observations that have been made on the testis, and its secretion, the semen, are mostly of a fragmentary nature. A large proportion of the chemical constituents of the organ is composed of proteids, or substances closely allied to proteids, of which the most important is nuclein. In addition a large number of extractives, both nitrogenous and non-nitrogenous, have been found. The following is a brief *résumé* of the chief observations that have been made.

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[1] E. Freund, *Wiener med. Jahrb.* 1886, p. 335.

[2] Budde, *Ueber das Fibrinferment*, Würzburg, 1889.

Sertoli[1] found that a watery extract of the fresh organ had an alkaline reaction; Treskin[2] found it had an acid reaction. The acidity is probably due, however, as in so many organs, to the commencement of post-mortem changes of the nature of putrefaction, and this view of the case is supported by the fact that Treskin found leucine and tyrosine to be present.

The proteids present are serum-albumin and a globulin precipitable by saturation with sodium chloride (Sertoli). Nuclein is present in abundance.

The extractives present are leucine and tyrosine (probably produced by post-mortem changes), lecithin, cholesterin, and fat (Treskin); creatine (Schottin[3]), inosite (Schottin, Külz[4]); and, in a case of diabetes, glycogen (Grohe[5]); adenine, xanthine, hypoxanthine, and guanine (Schindler[6]).

The salts present appear to be chiefly chlorides of sodium and potassium (Treskin).

The greater number of the above observations have been made on the testes of the lower animals, bull, dog, &c.

Semen.—This is the secretion of the testis, generally mixed with the secretion of the prostate. It is a whitish, viscid fluid, containing innumerable spermatozoa, which originate from the cells of the tubules of the testis.

While alive the tail of the spermatozoon exhibits lashing movements, akin to those of a cilium, by means of which locomotion is accomplished. This power is retained for hours, or even days, in the alkaline fluids of the body, but it is destroyed by weak acids, and by all strong reagents like alcohol, chloroform, strong alkalis, &c. The movement is stopped by cooling to 0° C., and also by a temperature over 53° C. The latter temperature appears to coagulate the protoplasm and quite kills the spermatozoon.

The chief chemical constituent of the spermatozoa is nuclein (Miescher[7]); this forms an external coating to the head, and within it are proteid matters. Miescher ascribes the formula $C_{29}H_{49}N_9P_3O_{22}$ to the nuclein obtained from the semen of the bull. In containing no sulphur this nuclein differs from that obtained from pus-corpuscles. By mixing semen with 10 to 15 per cent. sodium chloride solution, the outer portion of the spermatozoa swells, and thus a slimy, jelly-like mass is obtained. The nuclein of the outer covering of the spermatozoa does not appear to be in combination with a proteid, but with a base called *protamine*, to which Piccard,[1] from an examination of its platinum compound, has ascribed the formula $C_{16}H_{32}N_9O_2$. Another organic substance akin to a proteid was described in spermatozoa by Miescher; it was found to contain 4 per cent. of sulphur.

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[1] Sertoli, *Gazz. med. veterinaria*, anno ii. Milano, 1872; Hoppe-Seyler's *Physiol. Chem.* p. 773.

[2] Treskin, *Pflüger's Archiv*, v. 122.

[3] Schottin, Hoppe-Seyler's *Physiol. Chem.* p. 773.

[4] Külz, *Sitzungsb. d. Gesellsch. zu Beförd. d. Naturwiss. zu Marburg*, 1876, No. 4.

[5] Grohe, W. Kühne, *Arch. f. pathol. Anat.* xxxii.

[6] Schindler, *Zeit. physiol. Chem.* xiii. 438.

[7] *Verhandl. d. naturforsch. Gesellsch. in Basel*, vi. 138.

Next to nuclein and proteids the chief organic substance present in spermatozoa appears to be lecithin (Diaconow [2]).

Cholesterin and fat are also fairly abundant. Miescher gives the following percentages for the spermatozoa of the salmon :—

Nuclein	48·68
Protamine	26·76
Proteids	10·32
Lecithin	7·47
Cholesterin	2·24
Fat	4·53

In addition to these, small quantities of the other extractives already mentioned as being obtainable from the testis are present.

The crystals generally described as Charcot's crystals (p. 303) are said to form in human semen on evaporation (Böttcher [3]). Schreiner[4] considers these crystals to consist of the phosphate of a base of which the formula is C_2H_5N, and to which he gave the name *spermine*. This substance appears to be identical with the base called ethylenimine, which can be prepared artificially from ethylenediamine hydrochloride.[5]

The name *spermatin* has been given to the mucin-like substance in semen (Vauquelin, Kölliker) (*see* p. 145).

The prostatic secretion.—This secretion is slimy, opalescent, and in man and the dog of a neutral or alkaline reaction. According to Buxmann[1] its composition is—

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[1] *Ber. d. deutsch. chem. Gesellsch.* vii. 1714.

[2] Diaconow, Hoppe-Seyler's *Med. Chem. Unters.* ii. 221; iii. 405.

[3] *Arch. f. pathol. Anat.* xxxii. 525.

[4] *Liebig's Annalen*, cxciv. 68. In the account of the crystals on p. 303 the formula is incorrectly given.

[5] Ladenburg and Abel, *Ber. d. deutsch. chem. Gesellsch.* xxi. 758.

Water	98·5	per cent.
Proteids	0·45 to 0·92	„
Salts	1·0	„

The most abundant salt present is sodium chloride; potassium salts, sulphates, and phosphates also occur.

Prostatic calculi, of which the most important constituent is calcium phosphate, may occur (Paulizky,[2] Iversen[3]).

OVARY

The connective-tissue element of this organ is very large and yields chiefly gelatin and mucin. Proteids and nuclein are derived principally from the ova and other cells present.

The *corpora lutea* are composed of cells, and are coloured by a yellow pigment called lutein. This was first described by Thudichum;[4] this observer was also the first to point out that this pigment is distinct from hæmatoidin or bilirubin (a derivative of hæmoglobin), which is often also present.

Lutein is one of the class of pigments known as lipochromes, and other members of the same group occur in the blood (p. 254), egg-yolk, retina (p. 464), adipose tissue, &c. Lutein shows two absorption bands, one well marked between b and F, but nearer the latter; the other less well defined between F and G. (For Ovarian Cysts see p. 352.)

THE EYE

The outer coat of the eye, the sclerotic, with the cornea, which is continuous with it, has been described with the connective tissues. The middle coat, the choroid, is the vascular coat of the eye; the connective tissue corpuscles are, however, pigmented; the same is true for the cells of the iris; the pigment present is probably the same as fuscin contained in the hexagonal pigment-cells of the retina. The retina has already been described in the chapter on epithelium (p. 458).

The aqueous humour is lymph, and has been described under that heading (p. 350); the vitreous humour is jelly-like connective tissue, and will be found described under that heading (p. 467). The crystalline lens is the only part of the eye that still demands description.

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[1] Buxmann, *Beiträge zur Kenntniss des Prostatasaftes. Diss.* Giessen, 1864.

[2] Paulizky, *Diss.* Berlin, 1857.

[3] Iversen, *Maly's Jahresb.* 1874, p. 358.

[4] Thudichum, *Centralbl. med. Wiss.* vol. vii. 1869, p. 1.

The lens.—The crystalline lens of the eye is composed of many layers of fibres which are in origin elongated epithelial cells. It is enclosed by a capsule which is homogeneous, and resembles elastin in its insolubility.

The specific gravity increases from the centre outwards from 1194 to 1076 (Chevenix[1]); the refractive index also increases in the same way. The reaction of the lens is alkaline.

The following are the results of analyses made by Laptschinsky:—[2]

Water	63·50
Solids	36·50
Proteids	34·93
Lecithin	0·23
Cholesterin	0·22
Fats	0·29
Salts	0·82

The proteid present is a globulin; albumin is absent. The name globulin was first given to this proteid, and afterwards extended to include other proteids which form the well-defined class we call globulins. The name *crystallin* was then given to this particular globulin by Berzelius. Hoppe-Seyler describes crystallin as very like vitellin in its properties. It coagulates on heating it to 70° C. Laptschinsky speaks of this proteid as being fibrino-plastic; Kühne says this is not the case.

Cataract.—This may be due to the formation of vacuoles in the lens-fibres in cases of diabetes; this is the condition produced in frogs by injecting sugar into the circulation.

The opacity produced in the lens after death is caused in a similar way, and is not due to the coagulation of proteid, such as occurs in the *rigor mortis* of muscles.

Ordinary senile cataract is, however, a fatty degeneration, and the opacity appears to be chiefly due to the deposit in a crystalline form of cholesterin in the lens; at the same time the proteids are diminished in quantity. Cahn[3] gives the following percentages of dry residue in a case of cataract:—

Proteids	85·37
Cholesterin	4·55
Lecithin	0·803
Fat	1·19
Salts	3·86

Calcareous salts are said to be occasionally deposited in the lens.

[1] Kühne, *Lehrbuch*, p. 404.

[2] Laptschinsky, *Pflüger's Archiv*, xiii. 631.

[3] Hoppe-Seyler's *Physiol. Chem.* p. 692.

THE EAR

The pinna consists of elastic tissue (p. 473) covered by skin. The external auditory meatus is composed of hyaline cartilage (p. 481). The composition of perilymph and endolymph (p. 351), and otoliths (p. 496) has already been described. Beyond this there is as yet no chemical knowledge of this organ.

THE SKIN

The epidermis is a stratified epithelium ; its surface layers are horny in nature. This is especially marked in nails, hoofs, horns, and hairs. (For Keratin, or horny material, *see* p. 452.)

The deeper layers of the epidermis (Malpighian layer) are protoplasmic.

The true skin is composed of fibrous tissue (*see* p. 467).

The secretions of the skin will be taken later with other secretions of the body.

In *ichthyosis* there is a great increase in the horny layer of the epidermis: there is also found a quantity of fat, cholesterin, and hippuric acid. In *pellagra* fat and cholesterin are also found, with a little leucine and tyrosine. In both diseases the ash of the skin contains much silica.

PART IV

ALIMENTATION

CHAPTER XXVI

FOOD

FOODS are the substances which are required for the nutrition of the body. It has been calculated that a man of average weight loses about 1000 grammes of matter daily; this passes out in the expired air, the urine, sweat, fæces, and other excretions; the substances that pass out are comparatively simple bodies, like water, carbonic acid, and urea, formed in the chemical decompositions always going on in living matter.

Food is necessary to replace this waste if the body-weight is to remain constant; but the substances taken in in the form of food undergo many changes before they ultimately become a constituent part of the body. The changes are partly of a physical nature, such as mastication in the mouth, a thorough mixing by the peristalsis of the stomach, and solution in the watery secretions of different parts of the alimentary tract; these same secretions also fulfil a much more important function, namely, that of causing chemical changes in the food, converting insoluble into soluble, indiffusible into diffusible substances. These two sets of changes, physical and chemical, constitute what is called digestion. Absorption follows digestion; that is, the products of digestion pass through the walls of the alimentary canal into the blood or lymph circulating there. The blood- or lymph-stream carries the absorbed products to the tissues, which take them and make them part of themselves: this is assimilation. In some cases, however, assimilation may not occur immediately, but an organ like the liver may intercept such an absorbed material as sugar, and store it (in the form of glycogen), giving it out by degrees as it is wanted.

Before we can study the chemical processes concerned in digestion, absorption, assimilation, and nutrition, it is necessary that we should be acquainted with the raw materials, the foods, on which the digestive juices act.

THE PROXIMATE PRINCIPLES OF FOOD

If we examine the food-stuffs, such as milk, eggs, meat, and vegetables, we find that they are mixtures of various inorganic and organic materials, which are named proximate principles; and, moreover, the chief proximate principles of food are in the main the same as the chief proximate principles of the body they are destined to build up. They may be classified as follows :—

INORGANIC		*Water.* *Salts*, e.g. chlorides, phosphates, carbonates of sodium, potassium, calcium, &c.
ORGANIC	Nitrogenous	*Proteids*, e g. albumin, myosin, casein, &c. *Albuminoids*, e.g. gelatin, chondrin, nuclein, &c. *Simpler nitrogenous bodies*, lecithin, creatine, &c. *Iron-containing compounds.*[1]
	Non-nitrogenous	*Fats*, e.g. cream, fats of adipose tissue. *Carbohydrates*, e.g. sugar, starch, &c. *Simpler organic bodies*, e.g. alcohol, vegetable acids and salts, &c.

Liebig inaccurately divided the organic foods into assimilable or plastic (proteids) and combustible or respiratory (fats and carbohydrates) ; we, however, now know that all varieties of food are both assimilable and respiratory.

Water.—It is recognised as a matter of every-day knowledge that a good water supply is essential to the health and well-being of the community. The water used for drinking must be clear, odourless, and colourless. It must not be contaminated with sewage, nor with the pathogenic bacteria apt to occur in sewage. For the purposes of safety it should be filtered through an efficient filter, or, better still, boiled before it is consumed. Distilled water is insipid ; rain water has the same disadvantage ; the softness of water, i.e. its freedom from salts, deprives it of its pleasant taste. The salts of spring or river water vary immensely according to the rock through which the river or spring passes.[2] When the salt (magnesium sulphate, iron salts, &c.), or gas (carbonic acid, sulphuretted hydrogen, &c.) contained in any special spring, is accentuated either in quantity or quality, the water is often found to be useful as a therapeutic agent. Pure water alone, without any specially dissolved salt, is also a most useful addition to the physician's stock of remedies. The subjects of water analysis, of water as a therapeutic agent, and of mineral waters are obviously too large to be treated of in a work on physiology.

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[1] There is some doubt whether inorganic compounds of iron are absorbed (*see* p. 800) ; iron-containing foods are therefore classified with the organic proximate principles.

[2] A good drinking water should not contain more than twenty degrees of hardness, i.e. twenty parts of lime in 100,000 of water.

Water is taken into the body, not only as water pure and simple, but all other forms of food, and especially beverages, are composed of water mixed with something else. Even the solid foods contain large proportions of water; meat, for instance, contains about 75, bread 37, milk 86, eggs 74, potatoes 75 per cent. of water.

Inorganic salts.—The importance of these agents in nutrition has been already dwelt on; they are daily excreted in certain amounts and must be daily replaced by the same or approximately the same amount; and the enumeration of the salts of the body (p. 60) is also an enumeration of the salts of the food. Sodium salts, especially the chloride, are essential. About twenty grammes of sodium chloride is in the mean taken *per diem*, partly in the articles of diet themselves, but mostly in a separate form as a condiment. This salt doubtless supplies the chlorine for the acid of the gastric juice. Potassium salts are found more abundantly in muscle, nerve, and other solid structures of the body, and are especially contained in meaty foods and in potatoes. Calcium salts, particularly the phosphate and carbonate, are more especially necessary for bone and tooth, but are also universally distributed in the tissues, though in smaller proportions; these salts are chiefly derived from milk, eggs, cereals, and other vegetables. Iron is found not only in hæmoglobin, but also in the organs, such as liver and spleen, and in most of the fluids of the body, in milk, in eggs, and in many vegetable foods. Probably the iron absorbed from the alimentary canal is furnished wholly by organic compounds of iron formed either during plant life or during the life of other animals, being there again ultimately derived from plants. Bunge[1] terms these organic compounds of iron *hæmatogens*. The following table compiled by Beaunis[2] gives the percentage composition of the ash of various foods:—

Food	Potash	Lime	Magnesia	Soda	NaCl	Iron oxide	P_2O_5	SO_3	Silica
Milk . . .	23	17	2	7	4·7	0·47	28	0·05	0·06
Muscle . .	39	1·8	3·8	4·8	1·5	1	46	0·3	—
Brain. . .	34	0·7	1·2	10·4	4·7	—	48	0·75	0·4
White of egg	27	3	2·7	12	39	0·5	3	1·7	0·3
Yolk of egg	11	13	2·2	1	9	2	60	—	0·6
Wheat . .	27	2	6·6	0·4	—	1·3	62	—	—
Barley . .	20	1·6	7	—	—	2	38	—	29
Potatoes .	51	3	13	—	2·4	—	12	6·5	7
Lentils . .	35	6	2·4	13	4·6	2	36	—	—

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[1] Bunge's *Physiol. Chem.* transl. by Wooldridge, 1890, p. 100. Hæmatogen in most cases appears to be a compound of iron and nuclein.

[2] *Physiol. humaine*, i. 621.

Proteids.—These form the most abundant source of nitrogen to the body, and they are found in larger quantity in animal than in vegetable foods. The chief animal proteids used in food are the myosin of flesh, the casein and albumin of milk, the proteids of egg, and of blood. The chief vegetable proteids are glutin, vegetable myosin, and other vegetable globulins. Gelatin has also a certain nutritive value, but an animal fed on gelatin, to the total exclusion of proteids, wastes.

Carbohydrates, on the other hand, are derived chiefly from vegetable foods; the most important are starch, cane sugar, and grape sugar. The carbohydrates found in animal food are lactose in milk, glycogen in liver and muscle, and inosite in muscle, and other organs. Cellulose, gums, and mucilages are of little or no use in nutrition.

Fats.—The fat of adipose tissue consists of olein, stearin, and palmitin. The fat of milk contains certain lower glycerides in addition. The vegetable oils consist chiefly of olein and palmitin.

Vegetable acids and salts of those acids.—Oxalic, tartaric, citric, and malic are the most important of this group. In the body they are converted into carbonates.

THE PRINCIPAL FOOD-STUFFS

We do not actually use as foods the various organic proximate principles in the pure condition; it is necessary that in a suitable diet these should be mixed in certain proportions, and in nature we find them already mixed for us. In milk and in eggs, which form the exclusive food-stuff of young animals, all varieties of proximate principles are present and mixed in suitable proportions; hence these are spoken of as perfect foods. Eggs, though a perfect food for the developing bird, do not form a perfect food for mammals, as they contain too little carbohydrate. In most vegetable foods carbohydrates are present in excess, while in most animal foods the proteids are predominant; hence in a suitable diet these should be mixed in proper proportions. The food-stuffs we shall consider fully are milk, eggs, meat, bread, various flours, and seeds of plants used as food, and in conclusion certain accessories of food, such as alcoholic beverages and other stimulants and condiments. The table on the next page gives at a glance the percentage composition of the principal food-stuffs.[1]

Milk

Milk is a secretion which is characteristic of mammals. The acini of the mammary glands are during periods of non-lactation lined by flattened epithelium. During lactation, which begins after the birth of

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[1] From McKendrick's *Physiology*, ii. 9.

Food-stuffs	Water	Proteids	Starch	Sugar	Fat	Salts
Bread . . .	37	8	47	3	1	2
Wheat flour . .	15	11	66	4·2	2	1·7
Oatmeal . . .	15	12·6	58	5·4	5·6	3
Rice	13	6	79	0·4	0·7	0·5
Peas	15	23	55	2	2	2
Potatoes . . .	75	2	18	3	0·2	0·7
Milk	86	4	—	5	4	0·8
Cheese . . .	37	33	—	—	24	5
Lean beef . .	72	19	—	—	3	5
Fat beef . . .	51	14	—	—	29	4
Mutton . . .	72	18	—	—	5	5
Veal	63	16	—	—	16	4
White fish . . .	78	18	—	—	3	1
Salmon . . .	77	16	—	—	5·5	1·5
Egg	74	14	—	—	10·5	1·5
Butter.	15	—	—	—	8·3	2
Oats	12	10	57	11·2 (cellulose)	—	3
Hay	13	9	41	27 ,,	—	7
Rye straw . .	14	4	35	40 ,,	—	6
Red clover . .	78	3·5	8	8 ,,	—	2

the offspring, the cells are larger and are continually undergoing a fatty degeneration. They disintegrate, and the fat-globules so liberated float in a clear liquid which is also secreted by the cells from the lymph circulating in the gland.

The mammary glands of male animals are inactive; there have, however, been exceptional cases in the human species, as well as among lower animals, in which a secretion has been obtained from the mammæ of the male.[1]

The mammary glands of new-born animals of both sexes secrete a small quantity of milk for a few days; it is popularly termed 'witches' milk.' It does not differ qualitatively from the milk of the adult female, but it does differ quantitatively; but the few analyses that have been made show great variations in the amounts of the different constituents present (*see* Tables of Analyses).

The mammary glands themselves, apart from their secretion, do not seem to have been chemically investigated in a thorough manner. Their protoplasm, nuclei, and interstitial connective tissue have doubtless a similar chemical structure to that of these same constituents in other situations. Bert[2] found in the secreting glands a substance convertible by boiling with water or dilute acids into a sugar; and Landwehr[1] regards this substance as animal gum, which he here considers to be the mother-substance of milk sugar.

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[1] Schlossberger (*Ann. Chem. Pharm.* li. 481) analysed a specimen from a he-goat; he found the milk was alkaline and contained 9·6 per cent. of proteids and insoluble salts, 2·65 per cent. butter, and 2·6 per cent. lactose and soluble salts.

[2] *Gaz. hebdom.* 1879, No. 2.

The object which milk is intended to fulfil is that of supplying a food to the growing offspring. In the case of many animals domesticated by man (cow, goat, ass, &c.) the milk is collected and applied to other uses, namely, the feeding of man himself. Milk is, moreover, a perfect food: it contains members of all classes of proximate principles; its proteids are casein and an albumin; its carbohydrate is termed lactose or milk sugar; the fats constitute what we call butter; and the salts are chiefly phosphates and chlorides.

Microscopical appearances of milk.—The microscope reveals the fact that milk consists of two parts: a clear fluid, which may be called the milk-plasma, and a number of minute particles floating in it; the greater number of these are minute oil-globules, varying in size from 0·0015 to 0·005 millimetre in diameter;[2] the great majority are nearer the lower limit than the higher. In addition there are minute particles of casein and nuclein suspended in the fluid.[3] The milk which is secreted for the first few days after parturition, is somewhat imperfectly formed, and differs quantitatively from true milk. This is indicated microscopically by the presence of cells from the acini of the gland containing fat-globules which they have not yet liberated by disintegrating. These cells are termed *colostrum corpuscles*,[4] and the milk of the first few days of lactation is called colostrum.

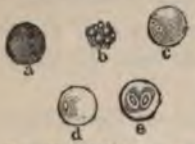

FIG. 81.—Cellular constituents of the Colostrum (Heidenhain). *a*, *b*, colostrum corpuscles with fine and coarse fat-globules respectively; *c*, *d*, *e*, pale cells devoid of fat-globules.

Milk is one of the most perfect emulsions; the fat-droplets never run together to form larger drops, but remain separately suspended in the albuminous milk-plasma. When milk is allowed to stand, a large proportion of the fat-globules rise to the surface, forming the cream; this can be hastened considerably by the use of the centrifuge. Cream is also an emulsion, but is much richer in fats than ordinary milk.

By shaking milk with ether without previous addition of caustic potash or acetic acid, the fat dissolves with some difficulty. Investigators have therefore concluded that each fat-globule is surrounded by shell of casein. Ascherson[1] showed that with an artificial emulsion of oil in an alkaline albuminous fluid the oil drops became coated with proteid; and Quincke[2] demonstrated the same fact, in which mucilage was used instead of albumin; each oil droplet had a gummy envelope. Casein certainly is not in solution in the milk-plasma or only in small quantities; if milk be filtered under pressure through a porous cell, the filtrate is free, not only from fat-globules, but from casein also.[3] The different methods employed for precipitating casein cause also a precipitation of the fat with it. In spite of these facts, however, Hoppe-Seyler[4] regards it as improbable that the greater part of the casein is present in the form of casings for the globules; he obtained the same amount of casein from cream as from portions of milk below the cream. The part of the casein around the globules is therefore, if present, imponderable. Hoppe-Seyler also states that it is not so difficult, as stated by the earlier observers, to remove the fat by simply extracting with ether; after the fat has been thus removed the fluid is still cloudy, but this is from the presence of the particles of casein and nuclein which were described by Kehrer.

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[1] *Pflüger's Archiv*, xl. 21. Thierfelden (*Ibid.* xxxii. 619) had previously recognised that this substance is not glycogen.

[2] Fleischmann, *Das Molkereiwesen*, Braunschwieg, 1876–9, p. 206.

[3] Kehrer, *Arch. f. Gynækologie*, ii. 1.

[4] Stricker (*Wien. Akad. Sitzungsber.* liii. Feb. 1, 1866) states that they show amœboid movements when placed on a warm stage at 40° C.

Reaction.—This is nearly always alkaline. Milk turns readily acid or sour as the result of fermentative changes, part of its lactose being transformed into lactic acid. In carnivora the milk is acid; in herbivora an amphoteric reaction is often observed, the acid sodium phosphate in it (H_2NaPO_4) turning neutral litmus paper red, and the alkaline sodium phosphate (Na_2HPO_4) turning it blue.

Specific gravity.—This is usually ascertained with the hydrometer. That of normal cow's milk varies from 1028 to 1034; when the milk is skimmed the specific gravity rises owing to the removal of the light constituent, the fat, to 1033 to 1037. This, fraudulent milk-vendors correct by the addition of water. Tables of specific gravities are published for the guidance of analysts, which indicate the purity of milk when skimmed and unskimmed, these numbers varying with the amount of water added.

Amount secreted.—The quantity of milk secreted by a woman is about 700 to 800 c.c daily, sometimes as much as a litre, and sometimes even more when the mother is suckling two or three children simultaneously. A good cow secretes six to seven litres daily.

Quantitative composition of milk.—The quantitative composition of milk varies in different classes of animals; it also varies with the state of nutrition, the constitution, and perhaps with the age of the individual. I am indebted for the following tables principally to Hoppe-Seyler's work on 'Physiological Chemistry.'

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[1] *Arch. f. Anat. u. Physiol.* 1840, p. 53.

[2] *Pflüger's Arch.* xix. 1879, p. 129.

[3] Zahn, *Pflüger's Arch.* ii. p. 298.

[4] Hoppe-Seyler, *Physiol. Chem.* p. 728.

1. *Human milk.*—The milk of new-born children (witches' milk).

Constituents	I[1]	II[2]	III[3]
Water	96·30	89·40	95·705
Solids	3·70	10·60	4·295
Casein	—	2·80	0·557
Albumin	—		0·490
Fat	0·82	1·40	1·456
Lactose	—	6·40	0·956
Salts	0·05		0·826

This milk like all human milk is alkaline. Witches' milk obtained from foals by Ammon[4] was acid, but this was probably from fermentation having set in.

2. *Human colostrum.*—The following analyses are by Clemm,[5] with the exception of the last, which is by Tidy: [6]

Constituents	4 Weeks before delivery	17 Days before delivery	9 Days before delivery	2 Days after delivery	—
Water	85·197	85·172	85·855	86·788	84·077
Solids	14·803	14·828	14·145	13·212	15·923
Casein		—	—	2·182	3·228
Albumin . .	6·903	7·477	8·073	—	
Fat	4·130	3·024	2·347	4·863	5·781
Lactose	3·945	4·369	3·637	6·099	6·513
Salts	0·443	0·448	0·544	—	0·335

The liquid is yellower than pure milk; it is strongly alkaline; it contains the colostrum corpuscles already described. It contains rather more solids than milk, a smaller quantity of casein, and a very much larger amount of proteid coagulable by heat; this is termed albumin in the above table; it is really globulin and albumin:[7] globulin is absent in normal milk.

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[1] Schlossberger and Hauff, *Ann. Chem. Pharm.* xcvi. 68.
[2] Gubler and Quévenne, *Gaz. méd. de Paris*, 1856, p. 15.
[3] V. Genser, *Jahrb. f. Kinderkrankheiten*, N.F. ix. 160.
[4] *Maly's Jahresb.* 1876, p. 118.
[5] R. Wagner, *Handwörterb. d. Physiol.* ii. 464.
[6] *London Hospital Reports*, 1867–8, p. 77.
[7] J. Sebelien, *Zeit. physiol. Chem.* xiii. 135.

3. *Normal human milk.*

Constituents	Clemm		Tidy	Biel[1]	Christenn[2]
	9 Days after delivery	12 Days after delivery			
Water	88·582	90·581	86·271	86·32 to 88·79	87·24
Solids	11·418	9·419	13·729	11·21 „ 13·68	12·76
Proteids	3·691	2·911	2·950	1·68 „ 3·15	1·90
Fat	3·532	3·345	5·370	2·59 „ 5·39	4·32
Lactose	4·298	3·154	5·136	5·79 „ 6·61	5·97
Salts	0·169	0·194	0·223	0·23 „ 0·34	0·28

On account of the difficulty in separating casein and albumin in human milk, they are given together in the above table. Other observers have, however, determined these two proteids separately; thus Tolmatscheff[3] found 1·28 of casein and 0·34 of albumin per cent.; Makris[4] found 1·8 to 4·8 of casein, and 0·7 to 1·7 of albumin per cent.

The gases of human milk have not been investigated.

Vernois and Becquerel[5] have investigated the influence of age on the quantitative composition of human milk, but with no specially interesting results. The same authors state that menstruation lessens the amount of lactose, but increases that of fat and casein.

The milk of blondes contains less casein and sugar, but more fat, than that of brunettes (L'Héritier[6]); Vernois and Becquerel agree with L'Héritier only so far as the casein is concerned; while Tolmatscheff could find no constant difference at all. It is indeed probable than other causes than complexion were at play in the cases observed by the French chemists just mentioned.

4. *Artificial human milk.*—Many recipes have been given to enable mothers who cannot suckle their children to prepare from cow's milk a milk like their own. Cow's milk is poorer in sugar, but richer in casein and butter, than human milk. Frankland gives the following table contrasting the milk of woman, ass, and cow :—

	Woman	Ass	Cow
Casein	2·7	1·7	4·2
Butter	3·5	1·3	3·8
Lactose	5·0	4·5	3·8
Salts	0·2	0·5	0·7

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[1] *Maly's Jahresb.* 1874, p. 168.

[2] *Dissertation*, Erlangen, 1877.

[3] *Med. chem. Unters.* ii. 272.

[4] *Dissert.* Strasburg, 1876, p. 31.

[5] *Compt. rend.* xxxvi. 188.

[6] *Traité de chimie pathol.* Paris, 1842, p. 683.

The following are the principal recipes for preparing artificial human milk :—[1]

(*a*) Cow's milk	600	grammes	
Water	339·5	„	
Cream	13	„	
Lactose	15	„	
Calcium phosphate	1·5	„	(Coulier)

(*b*) Heat half a pint of skimmed cow's milk to 35° ; add rennet. After ten to fifteen minutes, break up the curd finely, strain the whey off, and boil it, adding 110 grains of lactose. Strain again, and add it to two-thirds of a pint of fresh cow's milk and then two teaspoonfuls of cream. This should be freshly made every twelve hours (Frankland).

5. *Cow's milk.*—The following analyses of the milk and colostrum of the cow have been made :—

Constituents	Colostrum[2]	Milk[3]	Milk[4]
Water	78·7 per cent.	84·28 per cent.	85 to 86 per cent.
Solids	21·3 „	15·72 „	14 „ 15 „
Casein	7·3 „	3·57 „	3 „ 4 „
Albumin	7·5 „	0·75 „	0·3 „ 0·5 „
Fat	4·0 „	6·47 „	4 „
Lactose	1·5 „	4·34 „	4·5 „ 5 „
Salts	1·0 „	0·63 „	—
Specific gravity	1046 to 1065	1028 to 1034	—

Gases of cow's milk. In 100 volumes of milk the following proportions of gases were obtained at a metre pressure and 0° C. :- -

Gases	I[5]	II[5]	III[6]	IV[6]
Nitrogen	1·41	1·34	0·70	0·80
Oxygen	0·16	0·32	0·10	0·09
Carbonic acid	6·72	5 01	7·60	7·60

The reaction of cow's milk is weakly alkaline ; it, however, soon turns acid, and often shows an amphoteric reaction. The colostrum, as in human milk, is richer in albuminous solids than the fully formed milk. Cow's milk is whiter and more opaque than human milk; the casein differs somewhat from that of human milk (*see* p. 583). The quantity of contained gases is small, the nitrogen and oxygen being probably derived from admixture with air.

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[1] Charles, *Physiol. Chem.* p. 302.

[2] Fleischmann, *Das Molkereiwesen*, &c. p. 89.

[3] The mean of numerous analyses collected by Gorup-Besanez, *Lehrbuch*, 1878, p. 424.

[4] Similar mean calculated by Hoppe-Seyler. Some recent analyses of milk by Tatlock (*The Produce of the Dairy*, Glasgow, 1888) will be found in Dr. McKendrick's *Physiology*, vol. ii. p. 767.

[5] Setschenow, *Zeit. f. rat. Med.* x. 285.

[6] Pflüger, *Pflüger's Archiv*, ii. 166.

The evening milk is richer in solids than that of the morning.[1] Different species of cows differ in the richness of their milk.[2] A good milk goes with good feeding and general good health of the animals.[3] Excessive muscular work produces a milk very rich in albumin, so that, like colostrum, it coagulates on boiling (Fleischmann).

6. *The milk of other animals.*—The composition of the milk of a large number of other animals may be conveniently collected into a table:—

Animal	Percentages of							Remarks
	Water	Solids	Casein	Albumin	Fat	Lactose	Salts	
Dog[4]			14·6	—	13·16	1·2	—	Acid like that of all carnivora: rich in casein, fat, and calcium, poor in lactose; the latter is increased by starchy foods[5]
Goat	—	—		...	—	--	-	Closely resembles cow's milk except in smell and taste
Sheep[6]. . . .	82 to 84	15 to 17	4·7		4 to 8	3 to 4·6	0·6	Remarkable for the high percentage of fat
Buffalo[6] . . .	80·6	19·4	4·2	1·3	8·4	4·5	0·8	—
Camel[7] . . .	86·3	13·7	3·7		2·9	5·8	0·6	—
Horse[8] . . .	90	10	1·8	0·3	1·3	5·5	0·3	Alkaline, seldom neutral
„ [9] . . .	92·5	7·5	1·3	0·3	0·6	4·7	0·3	The casein is more like that of human than cow's milk. This is the milk used originally in the manufacture of koumiss and kephir in Russia, but that of the cow is employed in this country
„ [10] . . .	91	9	1·05		1·3	5·7	0·3	
Ass[11]	90·5	9·5	1·7		1·4	6·40		—
„ [12]	89	11	3·5		1·8	5	0·5	—
Pig[13]	83	17	7		7	2	1	—
„ [13]	81·8	18·2	5·3		6	6	0·08	—
Hippopotamus[14]	90	10			4·5	—	0·1	—

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[1] Struckmann and Bödeker, *Ann. Chem. Pharm.* xcvii. 150.

[2] *See* Fleischmann.

[3] Kuhn and Fleischer, *Landwirthsch. Versuchstationen*, xii. 405.

[4] Simon, *Die Frauenmilch*, Berlin, 1838; Dumas, *Compt. rend.* xxi. 707.

[5] Bensch (*Ann. Chem. Pharm.* lxi. 221) and Poggiale (*Gaz. méd.* (3), x. 259) lessened but did not abolish lactose by feeding on flesh only. *See* also Kemmerich (*Centralbl. med. Wiss.* 1866, No. 30) and Seubotin (*ibid.* No. 22).

[6] Vernois and Becquerel, *Union médicale*, 1857.

[7] Dragendorf, *Chem. Centralbl.* 1867, p. 78.

[8] Average of three analyses by Biel, *Maly's Jahresb.* 1864, p. 171.

[9] Soxhlet, *ibid.* 1878, p. 152.

[10] Weiske and Schrodt, *ibid.* 1878, p. 151.

[11] Gubler and Quévenne in Gmelin's *Handbuch*, viii. 267.

[12] Vernois and Becquerel.

[13] Leutner in Gorup-Besanez' *Lehrbuch*, p. 424.

[14] *Chem. Centralbl.* 1871, p. 149.

The Proteids of Milk

The proteids which occur in milk are two in number, one of which coagulates on the addition of the rennet ferment; this has, in accordance with general usage, been called *casein* in the preceding pages. It will be more convenient, however, to reserve the term casein for the clotted proteid, and to use the word *caseinogen* for its precursor in the milk.[1] This new word is fashioned on the same pattern as the words *fibrinogen*, the precursor of fibrin, and *myosinogen*, the precursor of myosin. The other proteid in milk is an albumin, which resembles serum-albumin in some particulars, but differs from it in others; hence we may give it the name *lactalbumin*. In addition to these two proteids, others have been described by various observers under the names lactoglobulin, lacto-protein, whey-proteid, &c.; peptone is also regarded by some as a constant constituent of milk. We shall consider the evidence on which these statements have been made, and finally arrive at the conclusion that these additional proteids do not exist normally in milk.

The coagulation of milk.—When milk is allowed to stand at the ordinary temperature, the chief change that it undergoes is a conversion of part of its lactose into fermentation lactic acid. This may be so excessive that the acid formed precipitates a portion of the caseinogen. This, however, must not be confounded with the formation of casein from caseinogen.

Sometimes, however, the milk undergoes true coagulation when allowed to stand; this is spoken of as spontaneous coagulation: it is not really spontaneous, but is produced by certain bacterial growths (aërobia) acting in the same way as does rennet, which is an unformed or chemical ferment; they, like rennet, have the power of converting the caseinogen into casein, much as fibrin-ferment converts fibrinogen into fibrin.

The way in which the clot is most readily formed is by means of rennet; this ferment is secreted by the stomach, especially by sucking animals, such as the calf. The pancreas secretes a milk-curdling ferment, and portions of other tissues, such as the testis, have the power of curdling milk also.

Preparation of rennet.—This is usually prepared from the fourth stomach of the sucking calf, which is dried, and then extracted with 5 per cent. solution of sodium chloride. From this the ferment may be precipitated by excess of alcohol, dried, and then dissolved in water when required. One part of such dry powder will cause curdling in 200,000 parts of milk.

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[1] Prof. Foster in the 5th edition of his text-book reserves the name *casein* for the proteid as it exists in the milk (caseinogen); he calls the clotted casein *tyrein*.

Hammarsten,[1] and later Friedberg,[2] showed conclusively that the active principle of rennet is not pepsin; that it requires for its efficient action the presence of calcium salts, especially of the phosphate; and that it will act in a weakly acid, neutral, or alkaline solution. It acts most readily at about the temperature of the body or a little higher (40° C.), and is destroyed by a temperature of about 70° C. The true ferment contained in rennet is termed *chymosin* by Friedberg,[2] and *rennin* by Foster;[3] this observer confirms the observations of Hammarsten, that pure pepsin has no coagulative power on milk.

When rennet is added to cow's milk the result is a coherent clot or curd, which expresses a clear yellowish fluid, the whey. The curd contains the fat entangled with the casein; the whey contains the albumin, salts, and sugar. In human milk the curd is composed of smaller flocculi, and a similar flocculent coagulation can be produced in cow's milk by previously boiling it, or by adding lime water, or soda water to it. This renders the milk less irritating to a delicate stomach.

Caseinogen.—This proteid may be precipitated from milk by the addition of acids, or by saturation with neutral salts, like sodium chloride or magnesium sulphate. In both methods the fat is entangled with the precipitate, and, when the saturation method is used, the adherent fat renders the precipitate so light that it floats on the surface of the concentrated saline solution. I have obtained caseinogen most satisfactorily by combining the two methods. Milk is first saturated with magnesium sulphate; the precipitate of caseinogen and fat is collected on a filter and washed from milk-serum by a saturated solution of the same salt. Distilled water is then added to the precipitate on the filter; this, in virtue of the salt adhering to the precipitate, dissolves out the caseinogen, and the fat is left on the filter, while the solution of caseinogen in dilute magnesium sulphate solution passes through the filter and is collected. From this solution the caseinogen is precipitated by means of excess of acetic acid; it is collected, thoroughly washed, dissolved in dilute alkali such as lime-water, and purified by repeated precipitation with acid and re-solution in alkali. If the caseinogen has been washed completely free from all calcium phosphate (a long and difficult process), the addition of rennet to the solution causes no formation of casein; but rennet *plus* calcium phosphate will produce almost immediate clotting at 40° C.; the addition of a little calcium chloride is also beneficial (Hammarsten [1]). Hammarsten himself does not use the word caseinogen, but speaks of both casein and its precursor as casein. A method introduced by him to show that casein precipitated by acid from milk (caseinogen) will undergo rennet coagulation (formation of true casein) is as follows: The caseinogen precipitated by acetic acid is well washed with distilled water until free from salts; it is mixed with powdered calcium carbonate and lime-water added till the pap-like mass so formed is just alkaline; a few drops of 0·5 per cent. phosphoric acid is added (to form calcium phosphate with the lime water), and a drop of rennet. The previously semi-fluid mixture now sets into a firm jelly-like mass.

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[1] *Maly's Jahresb.* 1874, p. 135.

[2] *Journ. of the American Chem. Soc.* May 1888, p. 15.

[3] *Text-book*, 5th edit. p. 419.

Caseinogen, usually spoken of as casein, is often compared to alkali-albumin. The latter, however, does not clot with rennet and is, unlike caseinogen, readily soluble in acids. Both are alike in precipitability by neutral salts, and in the fact that neither is coagulated on heating its neutral solution.

Caseinogen is like a globulin in the way it behaves to neutral salts. A solution of a globulin, however, coagulates when heated. A solution of caseinogen, such as that in dilute magnesium sulphate or sodium chloride solution, becomes a little cloudy at 70°, but this disappears when the solution is cooled if the heating has not been continued too long, but there is never any appearance of a flocculent precipitate.

Caseinogen as analysed by Chittenden[2] has the following percentage composition: C, 53·3; H, 7·07; N, 15·91; S, 0·82; O, 22·04. Danilewsky[3] has asserted that it is a mixture of two proteids—caseo-protalbin, partly soluble, and caseo-albumin, insoluble in hot 50 per cent. alcohol. Hammarsten[4] has shown that this peculiar behaviour of Danilewsky's preparations is due to their containing calcium phosphate; and this impurity depends on the use of hydrochloric acid as a precipitant, as this acid does not favour the removal of the salt as well as acetic acid. Both Hammarsten and Chittenden's analyses favour the view that caseinogen is a single proteid. Chittenden has also studied the products of digestion of caseinogen and casein, and finds that peptones are ultimately formed; certain intermediate bodies resembling the albumoses are also formed, and termed caseoses. Proto-caseose, hetero-caseose (produced only in small amounts), and deutero caseose can be separated, and correspond to the albumoses with similar names. An insoluble semi-gelatinous substance separates in the first stages of gastric digestion; it is only very slowly changed into soluble bodies, and is termed casein-dyspeptone.[5] Sebelien,[6] who has also prepared pure casein-peptone, states that it is optically inactive. All other proteids, so far as is at present known, are lævorotatory. The question whether the caseinogen of milk is in suspension or solution, or both, has been already discussed (p. 575).

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[1] The calcium salt must be a soluble one; other alkaline earths may be substituted for lime (Lundberg, *Maly's Jahresb.* 1876, p. 11; Ringer, *Journ. of Physiol.* 1890).

[2] *Studies from Lab. physiol. Chem. Yale Univ.* ii. 156.

[3] *Zeit. physiol. Chem.* vii. 433.

[4] *Ibid.* vii. 227.

[5] Chittenden, *Studies from Lab. physiol. Chem. Yale Univ.* iii. 66.

[6] *Bied. Centralbl.* 1889, p. 717

Casein.—This name should be restricted to the proteid formed by the action of rennet or rennet-like ferments from the caseinogen of milk. It is more insoluble than caseinogen in dilute alkalis. The casein of human milk, unlike that of the cow, separates in fine flocculi; when dried the powder formed is more yellowish than that from cow's milk. This corresponds to certain differences that have been described in the caseinogens. The caseinogen of human milk is more difficult to precipitate by acetic or carbonic acid, and more readily precipitated by magnesium sulphate than that of cow's milk. There are also stated to be differences in elementary composition.[1] Casein is the chief constituent of cheese.

There have been various explanations advanced to account for the action of lime-salts in favouring the coagulation of milk by rennet. Hammarsten is not inclined to believe that the lime combines with the caseinogen, but that the ferment produces the change in the caseinogen, and that the casein so formed will not separate out unless the calcium-salt is present. Green[2] suggests that there is some definite relationship between the ferment and the salt, resembling that which exists between pepsin and hydrochloric acid, and that the ferment cannot act without the presence of its inorganic ally. Ringer[3] finds that casein dissolved in lime-water separates out as a curd on the addition of calcium chloride; this curd is more soluble in cold than hot water, so resembling other lime-salts. Whether, however, casein normally formed by rennet is nothing more than a caseate of lime must be for the present regarded as uncertain.

Lactalbumin.—After the precipitation of caseinogen by magnesium sulphate, this proteid is left in solution. It can be incompletely precipitated from this solution by saturation with sodium sulphate. It coagulates between 70° and 80°; in cow's milk, which I have specially examined, at 77° C. It is not separable, like serum-albumin, into several proteids by fractional heat-coagulation. It moreover is coagulated by heat very slowly. The solution must be kept some hours at 77° before the proteid is entirely precipitated. Its specific rotatory power[4] is $(\alpha)_D = -36°$. Its elementary analysis gives the following percentages: C, 52·19; H, 7·18; N, 15·77; S, 1·73; O, 23·13; it thus differs from serum-albumin in specific rotatory power, in its high percentage of sulphur, and in its solubilities. The scum which forms on the top of milk when it is boiled is probably in part produced by the coagulation of the lactalbumin by heat; this carries to the surface a little caseinogen and fat. If the scum of boiled milk be removed, another forms, and this may be repeated many times in succession. The contact with the air thus appears to be of influence in causing the solidification which results in the formation of the scum; it may be because evaporation is more rapid from the surface exposed to the atmosphere. The boiling of milk before it is used as food is advantageous in two ways: (1) All germs of disease are destroyed; (2) the gastric juice, in virtue of its rennet, causes a flocculent, not a bulky precipitate.[1] These advantages quite outweigh any slight difficulty of digestibility which is alleged to occur (Raudnitz).[2]

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[1] Bredert and Schröter, *Centralbl. f. Agricultur-Chemie*, 1888.

[2] *Journ. of Physiol.* viii. 371.

[3] *Proc. Physiol. Soc.* 1890, p. iv.

[4] J. Sebelien, *Maly's Jahresb.* xv. 184.

Lactoglobulin.—Sebelien states that after removal of the caseinogen by saturating milk with sodium chloride an additional precipitate is obtained by saturating the filtrate with magnesium sulphate: this precipitate he considers to consist of a globulin which he calls lacto-globulin. There is no doubt Sebelien has here fallen into an error; for double saturation with the two salts just mentioned will precipitate albumins (*see* p. 246), and he has mistaken the precipitate of lactalbumin so produced for a globulin. A solution of the precipitate produced by saturating milk with magnesium sulphate never coagulates on boiling in the specimens I have examined; globulins are therefore absent, though doubtless a globulin is present, as Sebelien states, in colostrum.

Whey-proteid (*Molken-Protein*) (Hammarsten).—Rennet, according to Hammarsten, splits caseinogen into two proteids: one is the insoluble casein, the other a soluble proteid found in the whey, and equivalent to the lacto-protein of other investigators. Lacto-protein is stated by some investigators to be a peptone-like substance; and peptones and peptone-like substances are altogether absent both in fresh milk and in the whey of fresh milk. I have examined whey repeatedly and failed to find any peptone or proteose in it. On saturating it with magnesium sulphate a proteid is precipitated, and this appears to be Hammarsten's whey-proteid; its solutions do not coagulate on heating, and it differs from caseinogen in not being convertible into casein by means of rennet. I should suggest that caseinogen and whey-proteid should be included in a new class of proteids intermediate between globulins and albuminates.

Lacto-protein, proteoses, peptones.—The separation of proteoses and peptones from other proteids has only been possible since the introduction of ammonium sulphate as a reagent. The older method of estimating peptones (with which proteoses were confounded) was to acidify and heat, filter off the coagulated proteids, and the proteid left in solution was called peptone; this was precipitated by tannin and weighed. In reality this so-called peptone consists of the primary proteoses (proto- and hetero-proteose) formed by the hydrating action of the acidified hot water. This applies not only to milk, but to other fluids also. Milk, however, is a glaring example of how this mistaken method has led to mistaken results. Thus Struve[1] and Schmidt-Mülheim[2] describe peptone in milk; Dogiel[3] calls it lacto-protein, and J. Schmidt[4] speaks of it as hemi-albumose.

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[1] The reason that boiled milk will either not curdle at all, or more slowly than fresh milk, is that by boiling, a part of the dissolved calcium salt is precipitated as tricalcium phosphate.

[2] Raudnitz, *Zeit. physiol. Chem.* xiv. 1.

Milk and whey under no circumstances contain true peptone. After saturation with ammonium sulphate and filtering, the filtrate is always free from proteids.

Proteoses, such as the albumoses, may be identified by placing a liquid containing a mixture of proteids under alcohol for many months. All proteids but peptones and proteoses are by this means rendered insoluble; water, however, extracts peptones and proteoses from the precipitate, as these are not coagulated by alcohol. This solution is then saturated with ammonium sulphate; the precipitate, if any occurs, consists of proteoses; the proteid in the filtrate, if any is present, is peptone.

By this method of testing, fresh milk and whey from fresh milk are found to be free from proteoses. Sour milk or whey from sour milk contains a good quantity of primary proteoses. Koumiss and the similar substance kephir also contain abundance of proteoses, and, according to some, peptones also.[5] The so-called 'long-milk' of Upper Scandinavia also contains peptones (Sebelien).

Nuclein is found in small quantities in milk. The difference between this and the true nuclein of nuclei has been already pointed out (p. 203). The iron in milk is combined with nuclein (Bunge).

The Fats of Milk

The chemical composition of the fat of milk is very like that of adipose tissue (p. 487), with small quantities of the triglycerides of butyric, caproic, caprylic, caprinic, myristic, and arachic acids in addition.[6]

Milk contains also small quantities of lecithin, cholesterin, and a yellow lipochrome.

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[1] *J. prakt. Chem.* 1884, p. 73.

[2] *Pflüger's Archiv*, 1882, p. 287.

[3] *Zeit. physiol. Chem.* 1885, p. 602.

[4] *Diss.* Moskau, 1882.

[5] The above observations on the proteoses and peptones of milk have been published by Neumeister (*Zeit. Biol.* xxiv. 271) and Sebelien (*Zeit. physiol. Chem.* xiii. 135). I have independently made identical observations (*Journal of Physiology*, 1890). The subject of koumiss and kephir has quite a literature of its own. Full references will be found in the last ten or twelve volumes of *Maly's Jahresbericht*.

[6] Grunzweig, *Ann. Chem. Pharm.* clxii. 215. E. Wein, *Dissert.* Erlangen, 1876. Chevreul, *Recherches sur les corps-gras*, Paris, 1822. Lerch, *Ann. Chem. Pharm.* xlix. 212. Heintz, *Ibid.* lxxxviii. 300.

Cream.—This is simply the upper layers of milk allowed to stand, in which, therefore, the fat-globules are more numerous than in ordinary milk; the amount of fat in cream varies from 14 to 44 per cent.

Butter.—The fat-globules are broken up by mechanical agitation; the strokes of the churn must not, however, exceed thirty or forty per minute. About one-third of the original fat is left in the butter-milk. Butter contains small quantities of caseinogen and lactose, in addition to fat; sodium chloride is added. The fats of cow's butter consist of 68 per cent. of palmitin and stearin (solid fats), 30 per cent. of olein, and the remaining 2 per cent. of the specific butter fats (Bromeis).[1] In the winter time the solid fats are said to be increased (Fleischmann). The butter from human milk is richer in fluid fats than that made from cow's milk (Hoppe-Seyler).[2]

By exposure to the air, butter becomes rancid; this is partly due to a breaking up of the higher fats, and the production of lower fatty acids—formic, acetic, butyric, valerianic, &c.—partly to the formation of acrolein from glycerine, and partly, and according to Hagemann chiefly, to the formation of lactic acid from the lactose mixed with the butter.

Artificial Butter. Margarine.—The best forms of imitation butter are made from beef fat freed from the greater part of its stearin, and mixed with milk, colouring, and flavouring reagents. The worse forms of imitation butter are made from lard, tallow, olive oil, rape seed oil, &c. A good margarine contains 80 to 90 per cent. of fat and 5 to 6 per cent. of casein, salts, and pigment. Though not so assimilable as the butter from milk, it is a cheap and wholesome food.

Milk Sugar or Lactose

The characters of milk sugar which have been already described in the chapter on Carbohydrates (p. 102) are the same whether it is derived from the milk of the woman, cow, goat, and of all other animals from which it has been separated.

The formation of lactic acid from lactose by the activity of certain bacterial growths gives rise to the souring that occurs in stale milk. Hoppe-Seyler[3] has supposed from the rapid appearance of lactic acid in some cases, that the milk already contains a lactic acid enzyme, when it leaves the mammary glands.

Lactose by inverting ferments is changed into dextrose and galactose; these undergo on the addition of yeast the alcoholic fermentation, and so koumiss is prepared.

Extractives of Milk

There are a number of organic substances dissolved in the milk-plasma which may be called extractives, and of which a mere enumeration will suffice. The caseinogen may be precipitated by acid or by rennet; this carries the fat down with it, and the two together are then filtered off. The filtrate contains albumin, lactose, salts, and these extractives.

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[1] *Ann. Chem. Pharm.* xlii. 46.

[2] *Physiol. Chem.* p. 727.

[3] *Arch. pathol. Anat.* xvii. 417.

Among the latter, Ritthausen[1] separated a second carbohydrate of doubtful nature. Béchamp[2] obtained traces of alcohol, and acetic acid (0·021 to 0·2 gramme to the litre) from cow's milk; 800 c.c. of asses' milk yielded 30 c.c. of a distillate containing 3·5 per cent. of alcohol and 0·036 per cent. of acetic acid. The koumiss of Russia is made by allowing mare's milk to undergo the alcoholic fermentation. A similar substance, kephir, is made by adding the so-called kephir-grains to milk; the kephir-grains are masses of fungi and bacteria. Kephir contains less alcohol (0·5 to 1 per cent.) than koumiss (1 to 2 per cent.). Small quantities of lactic acid can generally be obtained from the freshest milk (Hoppe-Seyler[3]). Traces of urea have been described by several observers[4]; Commaille[5] found a trace of creatinine, and Musso[6] of a sulpho-cyanide.

The Salts of Milk

This subject has been worked at quantitatively by Bunge,[7] and with the following results: —

—	Human milk		Dog's milk		Cow's milk	Horse's milk
	I	II	I	II		
K_2O	0·78	0·71	1·41	1·68	1·76	1·04
Na_2O	0·23	0·26	0·80	0·69	1·11	0·14
CaO	0·33	0·34	4·53	4·28	1·59	1·23
MgO	0·06	0·06	0·19	0·21	0·21	0·12
Fe_2O_3	0·003	0·006	0·02	0·01	0·003	0·015
P_2O_5	0·47	0·47	4·93	4·67	1·97	1·31
Cl	0·43	0·44	1·62	1·8	1·69	0·31
Total ash per 1000	2·22	2·18	13·15	12·96	7·97	4·17

The chief acid present throughout is phosphoric acid; the chief base in human milk is potash; but this in the other animals in the list is second to lime; the lime in dog's milk is especially high.

In connection with the quantity of iron in the milk, Bunge[8] has made the interesting observation that although the other mineral constituents of milk are present in the same proportion as they are contained in the fœtal tissues, the quantity of iron in the milk is very much less. This is illustrated by the following analyses :—

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[1] *Journ. f. prakt. Chem.* N.F. xv. 348.

[2] *Compt. rend.* lxxvi. 654, 886.

[3] *Arch. f. pathol. Anat.* xvii. 433.

[4] Picard, *Thèse*, Strasburg, 1856; Lefort, *Compt. rend.* lxii. 190; and others.

[5] Commaille, quoted in Hoppe-Seyler's *Physiol. Chem.* p. 723.

[6] *Maly's Jahresb.* 1877, p. 168.

[7] *Diss.* Dorpat, 1874.

[8] *Zeit. physiol. Chem.* xiii. 399.

A hundred parts by weight of ash contain—

	In new-born dog	In dog's milk
K_2O	11·42	14·98
Na_2O	10·64	8·80
CaO	29·52	27·24
MgO	1·82	1·54
Fe_2O_3	0·72	0·12
P_2O_5	39·42	34·22
Cl	8·35	16·90
	101·89	103·80
Oxygen equivalent of the Cl	1·88	3·81
	100·00	100·00

The milk ash is rather richer in potash and poorer in soda than that of the new-born dog; this is easily explained by the fact that in the young animal the potash-rich muscle is increasing, and the soda-rich cartilage is diminishing. The higher percentage of chlorine is also explicable, as the chlorides not only serve to build up tissues, but also act largely as solvents in removing the end-products of metabolism through the kidneys. But the percentage of iron in the milk is only one-sixth of that in the fœtal tissues. The explanation appears to be that the fœtus obtains the greater part of its supply of iron before birth through the placental circulation, and stores it in the liver (*see* p. 552). Bunge has published analyses that show that a kilogramme of body-weight contains less and less iron as the young animal grows. Iron appears to pass to the child by the placenta rather than by the milk, because of the difficulties of absorbing iron by the alimentary canal, and the danger that hæmatogenous compounds may there become the prey of bacteria. Bunge regards it as probable that the large amount of iron which passes to the fœtus is not all derived from the mother's food during the relatively short period of pregnancy, but that a storage of iron occurs in the maternal organs even before the first conception, and this may explain the occurrence of chlorosis at the age of puberty.

Preservation of Milk

Milk may be preserved for a short time by boiling and tightly corking the vessel in which it is contained.

Antiseptics, of which the most commonly used are boroglyceride and boracic acid, may be added.

The milk may be concentrated at a low temperature and then preserved in hermetically sealed tins.

Most *condensed milks* have an antiseptic added to them, of which the most commonly employed is cane sugar. *Frozen milk* should be thoroughly thawed and well shaken before it is used as food, as the ice first formed carries a large percentage of the casein and cream to the surface.[1]

Cheese

Cheese is an important product of milk. The cheeses made in various parts differ according to the amount of cream mixed with the milk, and thus their percentage of fat varies. The essential constituent of cheese is the curd which is thrown down by rennet. All cheeses in addition contain a small admixture of lactose and a variable amount of salts. During ripening the fats and proteids both undergo decomposition, and thus free fatty acids are generally present. The following table[2] gives the percentage composition of some common cheeses :—

Cheese	Nitrogenous principles	Fats	Salts	Water
Cheshire	36·14	25·48	4·78	30 39
Gruyère	35·10	28·0	4·79	32·05
Roquefort	32·95	32·31	4·45	26·53
Cheddar.	28·4	31·1	4·5	36·0
Camembert. . . .	18·9	21	4·7	51·9

The Changes produced in Milk by Disease

It is a matter of every-day experience that the milk of a strong, healthy woman is more nourishing to the infant than that of weakly or sickly women. This has been supported by analyses.[3] Filhol and Joly[4] described a very abnormal case in which casein was altogether wanting. Bile-pigments and salts were described in milk in a case of jaundice by Frank,[5] but in similar cases subsequent observers[6] have failed to find them.

Certain drugs given to the mother pass into the milk, e.g. iodine, mercury (when given in large doses), arsenic, antimony, lead, zinc, and bismuth. Opium and morphia have never with certainty been found in the milk, but the milk of a mother dosed with opium is very fatal to the child.

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[1] Kaiser and Schmieder, *Bied. Centralbl.* 1887, p. 267.

[2] Charles, *Physiol. Chemistry*, p. 27.

[3] Décaisne, *Gaz. méd.* 1871, p. 317; Vernois and Becquerel, *Loc. cit.*

[4] Gorup-Besanez, *Lehrbuch*, p. 438.

[5] *Diss.* Giessen, 1879.

[6] v. Jaksch, *Prager med. Wochenschr.* 1880, No. 9.

The milk of animals, especially of the cow, is so important a food that it is essential it should be derived from healthy, well-fed specimens.

In cases of cattle plague the milk is found to contain blood.[1] The milk in cases of pearl disease should also be avoided, as the danger of infection from the presence of the bacillus of tubercle is greatly to be feared.[2] The milk from cases of foot and mouth disease and all affections of the teats of cattle is also injurious. Milk no doubt often acts as a carrier of infection; in certain cases it has been supposed that scarlet fever may be transmitted by its means; hence the prophylactic measure of boiling the milk before it is used. Concretions consisting chiefly of calcium carbonate with small quantities of phosphate and fat are occasionally met with in the teats of cattle.[3]

Blue milk[4] owes its colour, according to Fürstenberg, to triphenylrosanilin; it is doubtless produced by a bacterium and is said to produce diarrhœa. A purple-red micrococcus (*M. prodigiosus*) quickly grows in milk allowed to stand. Another bacterium (*B. synxanthus*) causes a yellow colour.

Milk Analysis

The estimation of the specific gravity of milk is important, as it furnishes a guide to the most frequent adulteration milk undergoes, namely, admixture with water, usually after removal of the cream.

The estimations of specific gravity, total water, solids, inorganic and organic may be carried out by the processes already described in Chapter II. The total solids should not be less than 11·5 per cent.

The microscopic examination of the milk should be then carried out most carefully. Starch grains, globules of other oils, and many other adulterations may be thus detected.

Quantitative estimation of the fat.—(1) An approximate estimation may be made by the thickness of the layer of cream. If milk be allowed to stand in a graduated vessel the cream should occupy 10 to 15 per cent. of the column.

(2) To 20 c.c. of milk add 20 c.c. of a 10 per cent. solution of potassium hydrate, and 100 c.c. of ether; shake the mixture vigorously; pour off the ether from the surface, and add more ether to the milk several times in succession until a fresh portion of ether shaken with the alkalised milk extracts no more fat. Mix together the ethereal extracts, and evaporate the ether on a water-bath in a weighed capsule; dry at 110° and weigh again; the increase of weight is the amount of fat in the 20 c.c. of milk. The normal minimum for fats in cow's milk is 2·5.

(3) Many optical methods have been described, the opacity of the milk depending on the number of globules present. In Donne's galactoscope a candle

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[1] Husson, *Compt. rend.* lxxiii. 1339.

[2] For analyses *see* Storch, *Maly's Jahresb.* xiv. 170.

[3] Fürstenberg, *Die Milchdrüsen der Kuh.* Leipzig, 1868.

[4] For recent observations on this subject *see* Reiset, *Compt. rend.* xcvi. 682, 745.

light is examined through a variable length of a column of milk until the light is occluded. By comparison with a standard the number of globules is calculated from the length of the column. In Vogel's method small measured portions of milk are added to 100 c.c. of water until a portion of the mixture examined in a vessel with parallel walls is opaque to light.

(4) Soxhlet's[1] method is one in which calculations are made from the specific gravity of the milk before and after removal of the fat.

Estimation of the proteids.—A large number of methods for estimating the proteids of milk have been devised from time to time. They consist essentially of the following: The casein is estimated by weighing either the curd produced by rennet, or the precipitate produced by acetic acid, after all fat has been removed from it by thorough extraction with ether. The albumin is estimated by weighing the precipitate produced by boiling after the removal of the casein or caseinogen. I have carefully examined the various methods that have been proposed, and arrived at the conclusion that J. Sebelien's[2] plan is the best. In outline it is as follows: The proteids are estimated, not by weighing them, but by estimating the nitrogen in the precipitates produced by various reagents; for this purpose admixture with fat does not matter; Kjeldahl's process of nitrogen estimation renders this method less formidable than at first sight it appears to be. The nitrogen multiplied by 6·37 gives the amount of proteid.

(1) Total nitrogen estimated in a known volume of milk.

(2) Nitrogen estimated in the precipitate produced by adding tannic acid to milk. This multiplied by 6·37 gives the total proteid.

(1) *minus* (2) is the non-proteid nitrogen: this is more abundant in colostrum than in milk.

(3) Nitrogen estimated in the precipitate produced by saturation with magnesium sulphate (casein + globulin[3]).

(4) Casein-nitrogen estimated in the precipitate produced by adding acetic acid to milk (approximate).

(5) Globulin-nitrogen; two estimations; maximal obtained by the difference between (3) and (4); minimal estimated in the precipitate produced by magnesium sulphate after separating the casein by saturating with sodium chloride. This is the only part of the process I regard as fallacious, for reasons already stated (p. 584).

(6) Albumin-nitrogen estimated in the precipitate produced by adding tannic acid to the filtrate after removal of the caseinogen by saturation with magnesium sulphate and filtering.

Estimation of the sugar.—A precipitate of caseinogen and fat is produced by adding acetic acid to a known volume of milk, and filtered off; the precipitate is washed with water, and the washings added to the first filtrate. This is then boiled with dilute sulphuric acid for half an hour, and the amount of dextrose so formed, estimated in it by means either of Fehling's solution or by the polarimeter.

Lactose may also be directly estimated by titrating the whey *plus* the washings of the curd (produced by rennet) with Fehling's solution: 10 c.c. of this solution is decomposed by 0·0676 gramme of lactose. Or the lactose may be estimated by the polarimeter without first converting it into dextrose; $(\alpha)_D = +59·3$.

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[1] *Zeit. d. landw. Vereins in Bayern*, 1882, p. 18. *See* also Egger, *Zeit. Biol.* xvii. 110; Schmöger, *J. f. Landw.* xxix. 129.

[2] *Zeit. physiol. Chem.* xiii. 135.

[3] As already shown (p. 583), globulin is absent from milk, though present in colostrum.

Uterine Milk

This is a creamy alkaline secretion of the uterine glands; it is especially abundant in ruminants. Its specific gravity is 1033 to 1040; it becomes acid quickly and coagulates. Microscopic investigation shows the presence of fat-globules, epithelial cells, nuclei, and *débris* of cells. The uterine milk of the cow has the following percentage composition: Water, 87·9; fat, 1·23; proteids and cells, 10·56; salts, 0·37. This secretion is doubtless nutritive to the embryo in the early stages of development.

Secretion of the Crop of Birds

Although milk is peculiar to mammals, John Hunter made the very remarkable observation that the mucous membrane of the crop of certain birds (doves) secretes a fluid very like milk for the first few days after the chick emerges from the shell. Cl. Bernard[1] compares this to milk, and believes that the birds feed their young with it during the first few days of their life. Leconte analysed this remarkable secretion, and found in it casein and salts 23·23, fat 10·47, and water 66·30 per cent. At other times the crop secretes a weakly alkaline fluid which has no nutritive or fermentative action.

Eggs

The ova of mammals are small and contain only a small amount of food material for the nourishment of the growing and dividing protoplasm; the close connection between fœtus and mother enables the former to obtain its nutriment from the latter. But in animals whose development *ab ovo* occurs outside the body of the mother, the necessity of a large store of food material is obvious; hence in oviparous animals the eggs are large, this increase of size being entirely due to the store of food material. In vertebrates the food material is at first continuous with the embryonic tissues; later it is placed in the yolk-sac which is attached to the primitive alimentary canal. As in the case of milk, the food thus provided for the developing offspring is diverted by man from its natural uses and employed by him as a food for himself; like milk, also, it is a highly nutritious food. The eggs of birds, and in this country especially the eggs of hens and ducks, are those particularly selected as a food-stuff. It will be, however, convenient to deal here, not only with hens' eggs, but to treat the subject from a more general standpoint.

Egg-shells consist in birds and some amphibia of a highly resistant keratinous material infiltrated with calcium carbonate and traces of magnesium carbonate and calcium phosphate.[2] The envelopes of the eggs of frogs and fishes are transparent and mucilaginous in consistency, being composed almost entirely of mucin.[1] In insects the egg-shells are chitinous. Egg-shells are formed in vertebrates not by the ovary, but by the walls of the passages by which they pass to the exterior.

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[1] *Leçons sur les propriétés physiol. des liquides de l'org* Paris, 1859, ii. 232.

[2] Analyses are published by Hilger (*Ber. deutsch. Chem. Gesellsch* 1873 p. 165), Wicke (*Ann. Chem. Pharm.* xcvii. 350; cxxv. 78), Brunnerstädt (*ibid.* xcv. 376).

Many eggs are coloured green, blue, red, brown, and so forth. The function of the pigment appears to be protective. The green and blue pigments are stated by Liebermann[2] to be derivatives of the bile-pigment.

The chief calcareous constituent of the egg-shells of birds is calcium carbonate. Dana and Buchanan state that the calcium carbonate excreted by polyps is absorbed as sulphate and converted first into sulphide and then into carbonate. Irvine and Sims Woodhead[3] therefore experimented with fowls, and fed them only on calcium sulphate; the birds, however, continued to lay eggs with normal shells. These observers also showed that fowls are unable to store up in their gizzard more lime as carbonate than is sufficient for the formation of the shells of two or three eggs, and that if lime be not procurable, either they will lay soft eggs or will cease to lay.

White of egg.—This is situated between the shell and the ovum proper or yolk; it forms an additional protection to the ovum, and is gradually absorbed by the yolk as development progresses. It is semi-fluid in consistency and pervaded by a meshwork of firmer, more fibrous material. This network is insoluble in cold or hot water, in salt solution, or in dilute acetic acid. It thus closely resembles the similar membrane enclosing the vitreous humour of the eye.

The semi-fluid material which fills the meshes is alkaline, and is especially rich in proteids. It has the following composition (Lehmann):

Water	82 to 88 per cent.
Solids	13·3 per cent. (mean)
Proteids	12·2 „ „
Sugar	0·5 per cent. (8 per cent. of dry residue) (Meissner)
Fats, alkaline soaps, lecithin, cholesterin	Traces
Inorganic residue	0·66 per cent.[4]

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[1] Wolfenden, *Journ. of Physiol.* v. 91.

[2] *Ber. chem. Ges.* xi. 606. The pigments of egg-shells have also been worked at by Sorby (*Proc. Zool. Soc. London*, 1875, p. 351), Wicke (*Göttingen. gelehrte Anzeig.* 1855, p. 314), and Krukenberg (*Verhandl. d. physik.-med. Gesellsch. Würzburg*, xvii. 109). A large number of pigments are described, but they have little physiological interest.

[3] *Lab. Reports of the College of Physicians, Edinburgh*, i. 62, ii. 122.

[4] The ash of white of egg analysed by Poleck (*Pogg. Annal.* lxxix. 155) and Weber (*Ibid.* lxxxi. 91) gave the following percentage results: K_2O, 27 to 28; Na_2O, 23 to 32; CaO, 1·7 to 2·9; MgO, 1·6 to 3·7; Fe_2O_3, 0·4 to 0·5; Cl, 25 to 28; P_2O_5, 3·7 to 4·8; SO_3, 1·3 to 2·6; SiO_2, 0·2 to 2; CO_2, 9 to 11. Nicklé's (*Compt. rend.* xliii. 885) found a trace of fluorine.

The proteids of white of egg fall into two classes—viz. (1) globulins, those precipitable by dilute acetic acid, carbonic acid, or saturation with magnesium sulphate or sodium chloride ; (2) albumins, those remaining in solution after the precipitation of the globulins. Egg-albumin, the most important of the proteids, differs from serum-albumin in not being precipitable by ether,[1] and in its specific rotatory power[2] $(\alpha)_D = -35{\cdot}5$. Hofmeister[3] has prepared it in a crystalline form by slowly evaporating a solution of it in half-saturated ammonium sulphate solution.

Corin and Berard[4] have separated the proteids by saturation with salts and fractional heat-coagulation. They name the proteids so obtained oviglobulin α (pp. at 57·5°), oviglobulin β (pp. at 67°) ; ovalbumin α (pp. at 72°), β (at 76°), and γ (at 82° C.).[5] Peptones and albumoses are absent in fresh eggs, but they appear and increase as the egg becomes stale.

Yolk of egg.—This substance is rich in the materials that go to form cells *plus* a considerable amount of fat. Microscopic investigation reveals the presence of two varieties of yolk-spherules—one kind yellow and opaque (due to admixture with fat and a yellow lipochrome), the other smaller, transparent, and almost colourless ; in addition there are smaller granules. These spherules are semi-crystalline, and correspond to the aleurone grains of plant-seeds, and the proteid of which they consist is called vitellin. Vitellin is a globulin, as it is insoluble in water ; it is, however, soluble both in weak and saturated solutions of sodium chloride, in the latter particular differing from other globulins. In the yolk, vitellin appears to be either in combination or intimately mixed with the phosphorised bodies lecithin and nuclein.

Vitellin may be prepared free from fat and the greater part of the lecithin by extraction with large quantities of ether. The residue consists of vitellin.

The proteids described by Valenciennes and Frémy[6] in the yolk of fishes' eggs and termed by them *ichthin*, *ichthulin*, and *emydin* are regarded by Hoppe-Seyler as doubtful chemical units, and are probably mixtures of vitellin with nuclein and lecithin. Nuclein was first described in yolk by Miescher.[7]

The constituents of the yolk may be thus enumerated :—

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[1] Schmidt, *Pflüger's Arch.* viii. 75.

[2] Gautier (*Compt. rend.* lxxix. 228) and Béchamp (*Ibid.* lxxvi. 1558) give rather a higher number for the albumin from the eggs of different birds.

[3] *Zeit. physiol. Chem.* xiv.

[4] *Arch. de Biol.* ix. 1.

[5] Haycraft (*Brit. Med. Journ.* vol. i, 1890) has recently criticised the method adopted in these researches.

[6] *Ann. de chim. et de phys.* (3), l. 129 ; *Ann. Chem. Pharm.* cxxvii. 188.

[7] *Med. chem. Untersuchungen*, iv. 502. *See* also p. 203.

(*a*) **Proteids :**
  Vitellin—the principal one.
  Albumin—in small quantities.
  Nuclein. The iron in the yolk is combined with this substance.
(*b*) **Fats :**
  Olein, palmitin, and stearin.
  A yellow lipochrome or lutein.
(*c*) **Carbohydrate :**
  Grape sugar in small quantities.
(*d*) **Other organic constituents :**
  Lecithin, originally described as protagon (*see* pp. 524, 527).
  Cerebrin (?).
  Cholesterin.
(*e*) **Inorganic salts :**
  The most abundant is potassium chloride. Weber gives the ratio $K_2O : Na_2O = 9{\cdot}05 : 4{\cdot}39$. Phosphates are also present.

The chemical changes that the yolk undergoes during development have been the subject of researches by Spallanzani, Prout,[1] Prévost and Morin,[2] Baudumont and St. Ange,[3] Burdach,[4] Fischel,[5] Pott,[6] and others. The inorganic constituents of the yolk, especially of phosphates, gradually diminish, building up the bones of the embryo.[7] Fischel found that peptones are formed as incubation goes on, but his method of estimating peptones is open to much question. The respiratory changes have been already described (p. 394).

Parke[8] gives the following quantitative analyses of the yolk of hens' eggs :—

—	Eggs not incubated	After 10 days' incubation	After 17 days' incubation
Water	47·192	57·308	44·787
Solids	52·808	42·692	55·213
Proteids . . .	15·626	14·201	13·942
Lecithin . . .	10·720	8·406	10·677
Fat	22·838	16·986	26·935
Cholesterin . .	1·750	1·281	1·461
Salts	0·965	0·910	1·338

Taking the hen's egg as a whole as 1000, 106·9 parts consist of shell, 604·2 of white, and 288·9 of yolk. The highly nutritious nature of both white and yolk as food will be evident from glancing at the various tables of analyses that have been given. Their nutritious value is great, as almost the whole of the substance is readily digested. One hen's egg is, according to Voit, equal to forty grammes of fat meat, or 150 grammes of milk. Raw eggs are more easily digestible than cooked eggs. The more an egg is cooked, the more insoluble do its proteid constituents become.

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[1] *Phil. Trans.* 1822, p. 377.

[2] *Ann. des sciences nat.* iv. 47.

[3] *Ann. chim. phys.* (3), xxi. 195.

[4] *Dissert.* Königsberg, 1858.

[5] *Zeit. physiol. Chem.* x. 11.

[6] *Landwirthsch. Versuchstat.* xxiii. 208.

[7] The shell does not alter (Prévost and Morin, Pott).

[8] *Med. chem. Untersuch.* ii. 209.

Meat.

This is composed of the muscular and connective (adipose) tissues of certain animals. These tissues have already been fully described, so that we have now only to consider them from the point of view of food. The food of many animals is not eaten; in some cases this is a matter of fashion, in others due to an unpleasant taste, such as the flesh of the carnivora are said to have, and in other cases (e.g. the horse) because it is more lucrative to use the animals as beasts of burden than as food.

Meat is the most concentrated, and most easily assimilable, of the animal nitrogenous foods. It is our chief source of nitrogen; its principal proteid is myosin. In addition to the extractives and salts contained in muscle, the flesh used as food always contains a certain percentage of fat, even though all visible adipose tissue be carefully cleaned off. The fat-cells are situated between the muscular fibres, and the amount of fat so situated varies with different animals.

The following table[1] gives the chief substances in some of the principal meats used as food:—

Constituents	Ox	Calf	Pig	Horse	Fowl	Pike
Water	76·7	75·6	72·6	74·3	70·8	79·3
Solids	23·3	24·4	27·4	25·7	29·2	20·7
Proteids and gelatin[2]	20·0	19·4	19·9	21·6	22·7	18·3
Fat	1·5	2·9	6·2	2·5	4·1	0·7
Carbohydrate	0·6	0·8	0·6	0·6	1·3	0·9
Salts	1·2	1·3	1·1	1·0	1·1	0·8

Meat thus contains four times the amount of proteid present in an equal weight of milk. In estimating the nutritive value of a food, one not only has to consider the amount of food material it contains, but whether it is difficult or easy of digestion. This subject will be more fully entered into in connection with the subject 'Digestibility of Foods,' where also a brief statement concerning the principal methods of cooking meat will be found (p. 609).

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[1] Munk's *Physiologie*, p. 260.

[2] The flesh of young animals is richer in gelatin than that of old; thus 1000 parts of beef yield 6, of veal 50 parts of gelatin (Liebig).

Salted meat loses a small proportion of its constituents, which pass out into the brine (0·01 albumin, 0·14 extractives, and 0·09 P_2O_5 per cent.; Voit).

Smoked meat.—The outer surface is hardened by the coagulation of the outer layer, certain matters in the smoke (creosote, &c.) acting as antiseptics.

Soups.—These contain the extractives of meat, a small proportion of the proteids, and the principal part of the gelatin. The gelatin is usually increased by adding bones and fibrous tissues to the stock. The presence of gelatin causes the soup when cold to gelatinise.

Beef-tea is an extract made by gradually and gently warming lean beef in water. Its stimulating effect is due to the extractives creatine, xanthine, hypoxanthine, lactic acid, and salts. It is nutritious only to a slight extent, as it contains mere traces of proteids, gelatin, and fats.

Liebig's Extract, and many other meat extracts now made, are concentrated beef-teas. Liebig's Extract contains 78 per cent. of solids, of which 61 are organic (extractives) and 17 inorganic. Its good effect in the sick-room is due to the extractives, which are stimulating, and to the salts, which are also stimulating; a simple solution of potassium phosphate is very refreshing.

Vegetable Foods

The chief distinction between animal and vegetable cells is the presence in the latter of an excessive amount of carbohydrate material, including an investing wall of cellulose; and this replaces to a large extent the original protoplasm (proteid) of the cells. Vegetable foods, then, speaking generally, are rich in starch, sugar, and cellulose, and comparatively poor in albuminous substances. The cellulose is indigestible or almost so; hence the fæces, which consist of undigested residues, are larger in volume in herbivora than in carnivora.

Vegetable proteid is, so far as analysis goes, practically the same as animal proteid. For some reason not yet understood, it is, however, not so easily digested; hence, even if a vegetable food contains as much nitrogen per cent. as an animal food, for purposes of nutrition it contains less.[1] The varieties and properties of the vegetable proteids are described in Chapter X (p. 131).

In the ash of vegetables the salts of potassium and magnesium are, as a rule, more abundant than those of sodium and calcium.

Cereals.—The average percentage composition of the cereals is given in the following table (Munk):—

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[1] The investigations of Rutgers (*Zeit. Biol.* xxiv. 251) seem to point to the fact that this is due rather to the admixture of vegetable proteids with indigestible substances than to any peculiarity in the proteids themselves.

Constituents	Wheat	Rye	Barley	Oats	Rice	Maize	Millet
Water	13·6	15·1	13·8	12·4	13·1	13·1	11·0
Albumin . . .	12·4	11·5	11·1	10·4	7·9	9·9	10·8
Fat	1·4	1·8	2·2	5·2	0·9	4·6	5·5
Carbohydrates .	67·9	67·8	64·9	57·8	76·5	68·4	66·8
Cellulose . . .	2·5	2·0	5·3	11·2	0·6	2·5	2·6
Ash	1·8	1·8	2·7	3·0	1·0	1·5	2·4

Flour is made from cereals and from other seeds by removing the husk and grinding the remainder. The best wheat flour is made from the white interior of the wheat grains, and contains the greater proportion of the starch of the grain, and most of the proteid. Whole flour is made from the whole grain *minus* the husk, and thus contains not only the white interior, but also the harder and browner outer portion of the grain. This outer region contains a somewhat larger proportion of the proteids of the grain. Whole flour thus contains 1 to 2 per cent. more proteid than the best white flour ; but it has the disadvantage of being less readily digested. Brown flour contains a certain amount of bran (the coating of the grains) in addition ; it is still less digestible, but is useful as a mild laxative, the insoluble cellulose mechanically irritating the intestinal walls as it passes along.

The best flour contains, or should contain, little or no sugar. The presence of sugar indicates that germination has commenced in the grains. In the manufacture of malt from barley, this is purposely allowed to go on.

Wheat flour when mixed with water forms dough, a sticky, adhesive mass. This is due to the formation of gluten ; and the forms of grain which are poor in gluten cannot be made into dough or bread (oats, rice, &c.). Gluten does not exist in the flour as such, but is formed on the addition of water from the pre-existing globulins (Martin ; *see* more fully p. 135).

Bread is made by cooking the dough of wheat flour mixed with yeast, salt, and flavouring materials. The yeast acting at the commencement of the baking, when the temperature of the oven is little above that of the body, forms sugar and dextrin from the starch, and then the alcoholic fermentation occurs. The bubbles of carbonic acid burrowing passages through the bread make it light and spongy. This sponginess enables the digestive juices subsequently to soak into it readily, and affect all parts of it. In the later stages of baking, the gas and alcohol are expelled from the bread, the yeast is killed, and a crust forms from the drying of the outer portions of the mass of dough. Other methods have been, or are, adopted for making dough light ; the leaven of the ancients was a piece of putrid dough ; baking powders are mixtures containing sodium bicarbonate, from which the carbonic acid is driven off during baking. White bread contains in 100 parts, 7 of proteid, 55 of carbohydrates (starch, dextrin, and sugar, the two last more abundant than in the flour), 1 of fat, 2 of salts, and the rest water. An adult would require daily about 1·6 kilo. of bread to supply him with the requisite amount of proteid ; this would, however, contain an overdose of carbohydrate.

Leguminous plants.—The meal of peas, beans, and lentils are rich in proteids, and are used by vegetarians as substitutes for meat. Potatoes are chiefly starchy. The percentage composition of the foods just mentioned is given in the following table :

Constituents	Lentils	Peas	Beans	Potatoes
Water	12·5	14·3	14·8	76·0
Proteids	24·8	22·6	23·7	2·0
Fat	1·9	1·7	1·6	0·2
Carbohydrates . .	54·8	53·2	49·3	20·6
Cellulose	3·6	5·5	7·5	0·7
Ash	2·4	2·7	3·1	1·0

It has been calculated that 4·5 kilogrammes of potatoes would be necessary daily to supply an adult with the requisite amount of proteid ; a bulk far too great for an ordinary alimentary canal to manipulate. In fact, unless a vegetarian diet is supplemented by some concentrated form of proteid, like milk, eggs, or cheese, it will be found impracticable, and the person who takes it will waste. Fermentative changes occurring in the alimentary canal, and giving rise to gases (carbonic acid, marsh gas, &c.) from the starch and cellulose, give rise to flatulence, which is a most serious drawback to vegetarianism.[1] Rice has not this particular disadvantage to such a marked extent.

It is well known that the inhabitants of certain countries (e.g. the coolies of India) are able to subsist on a smaller quantity of nitrogenous food than the average European. Recent experiments have shown that even Europeans can train themselves, gradually, to maintain bodily equilibrium for short periods on less than the fifteen grammes of nitrogen daily which has been hitherto supposed to be necessary.[2] The disadvantages of pure vegetarianism resulting from overloading the stomach and flatulence remain, however, unaltered. Looking at the table just given, it is seen that beans contain rather more nitrogenous material than beef : but experiments on man have shown that beans are a most unsuitable form of food when taken exclusively. It cannot be too often repeated that the digestibility of a food, as well as its percentage composition, must be taken into account in estimating its nutritive value. Prausnitz'[1] experiments with beans gave the following results: The fæces contained 18·3 per cent. of the food weighed as dry material, and 30·3 per cent. of the nitrogen undigested. Beans thus compare most unfavourably with bread, lentils, and other forms of vegetable food.

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[1] *See* Rutgers, *Zeit. Biol.* xxiv. 351.

[2] F. Hirschfeld, *Pflüger's Archiv*, xli. 588.

Green vegetables.—These are taken as a palatable adjunct to other foods, rather than for their nutritive properties. Their potassium salts are, however, abundant. Cabbage, turnips, and asparagus contain 80 to 92 water, 1 to 2 proteid, 2 to 4 carbohydrates, and 1 to 1·5 cellulose per cent. The percentage composition of the green foods of herbivora have been already given (p. 573), and the small amount of nutriment they contain accounts for the large meals made by, and vast capacity of the alimentary canal of these animals.

Accessories to Food

Alcohol.—Small quantities of the alcohol taken leave the body by the breath and urine as such; the greater amount is decomposed into simpler products (acetic, oxalic, carbonic acids, and water); the formation of these must give rise to a certain amount of bodily heat. It has been calculated that a man can burn off in his body two ounces of absolute alcohol daily. Alcohol is thus within narrow limits a food. It, however, lessens proteid metabolism by about 6 per cent., and thus ultimately leads to a diminution of the heat produced in the body. It is, moreover, a very uneconomical food; much more nutriment would have been obtainable from the barley or the grapes from which it was made. The value of alcohol used within moderate limits is not as a food, but as a stimulant, not only to digestion, but to the heart and brain.[2] The percentage of alcohol in liquors is as follows: Spirits, 50 to 60 or 65; port and sherry, 16 to 25; clarets and champagne, 5 to 13; porter and Bass' beer, 8 to 10; light beers 2 to 5. Various liquors are differently coloured and flavoured; some, like hocks, are acid; others are sweet from the presence of sugars and glycerine; port abounds in tannin, sherry and brandy in various ethers and alcohols; some, like champagne, are sparkling from excess of carbonic acid. Malt liquors contain bitter and other principles from the hop.

Condiments, like mustard, pepper, ginger, curry powder, are stomachic stimulants. Their abuse is followed by dyspeptic troubles.

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[1] *Zeit. Biol.* xxvi. 227.

[2] The reader interested in the subject of alcohol in diet should read Chapter VIII of Bunge's *Physiol. Chem.* (transl. by Wooldridge).

Tea, coffee, and cocoa.—These are stimulants chiefly to the nervous system. Tea, coffee, maté (Paraguay), guarana (Brazil), cola nut (Central Africa), bush tea (South Africa), and a few other plants used in various countries, all owe their chief property to an alkaloid called theine or caffeine ($C_8H_{10}N_4O_2+H_2O$); cocoa to the closely related alkaloid, theobromine ($C_7H_8N_4O_2$); coca to cocaine. These alkaloids are all poisonous, and, used in excess, even in the form of infusions of tea and coffee, produce over-excitement, loss of digestive power, and other disorders well known to the practical physician. Coffee differs from tea in being rich in aromatic matters; tea contains a bitter principle, tannin; to avoid the injurious solution of too much tannin, tea should only be allowed to infuse (draw) for a few minutes. Cocoa is a valuable food in addition to its stimulating properties, containing about 50 per cent. of fat and 12 per cent. of proteid.

I have not attempted to give references to the vast amount of literature published on the subject of alcohol. A few of the more recent papers on the subject will be found referred to in the chapter on alcohol in Bunge's book. I am indebted to the same book for the following references to papers on the constitution and physiological action of caffeine and allied alkaloids. Caffeine is trimethylxanthine (i.e. xanthine with three methyl groups introduced into its molecule), and can be prepared artificially (E. Fischer, *Liebig's Annalen*, ccxv. 253). Theobromine (the alkaloid contained in cocoa, and mixed with caffeine in guarana) is dimethylxanthine. Mono-methylxanthine is at present unknown. The physiological action of this series of substances has been studied by Filehne (*Du Bois Reymond's Archiv*, 1886, p. 72), and Kobert (*Arch. f. exp. Path. u. Pharm.* xv. 22).

CHAPTER XXVII

DIET

A HEALTHY and suitable diet must possess the following characters:—

1. It must contain the proper amount and proportion of the various proximate principles—proteids, fats, carbohydrates, salts, and water.

2. It must be adapted to the climate, age, and sex of the individual, and to the amount of work done by him.

3. The food must not only contain the necessary amount of elements, but these must be present in a digestible form.

The subject of diet is necessarily related to that of excretion. The object of the food is to repair the waste of the body; the amount of waste or loss must be known before the amount necessary for repair can be ascertained. The varying relations between income and expenditure, and the balancing of the two sides of the sheet, cannot here be conveniently studied in detail, but after our consideration of the urine and other excretions, we shall be then better able to consider the exchange of material or metabolism of the body in relation to nutrition. For the present we must content ourselves with stating very briefly the principles on which diets have been constructed, and this we can do most readily under the three heads enumerated above.

The relation between the proximate principles in a diet.—We have seen that a proteid contains carbon, hydrogen, oxygen, nitrogen, and sulphur, and it might be said that proteid *plus* water and mineral matter would supply a man with all the materials he wants, and the question will be asked, what is the use of the fats and carbohydrates, which only contain carbon, hydrogen, and oxygen? If we examine the materials that leave the body, we shall obtain an answer to this question. A man doing a moderate amount of work will eliminate, chiefly by the lungs in the form of carbonic acid, from 250 to 280 grammes of carbon *per diem*. During the same time he will eliminate, chiefly in the form of urea in the urine, about fifteen to eighteen grammes of nitrogen.[1]

In order to repair this loss, the daily food should contain, roughly, the same quantities of carbon and nitrogen, and the relation between carbon and nitrogen should be 250 to 15, or 16·6 to 1. The proportion of nitrogen to carbon in proteid, is, however, 53 to 15, or 3·5 to 1. Hence if a person lives entirely on proteid food, his diet will be incorrect in one of two ways: if the amount taken is adjusted to give the right weight of carbon, the nitrogen will be much too high; or if the amount taken is adjusted to give the right weight of nitrogen, the carbon will be much too low. In the first case, when the amount taken brings the quantity of carbon to the correct level, the food would be a bulky one, in fact so bulky as to be impracticable; 250 grammes of carbon would mean 500 grammes of proteid, and this would be obtainable in two to three kilogrammes (five to six pounds) of beef. This quantity of beef would contain much more nitrogen than the body has lost, and much more than the kidneys can excrete. In carnivorous animals, the capacity of the body for producing urea is greater than in man; but in man the accumulation of nitrogen and increased work of the kidneys, which are doing their best to get rid of the nitrogen, lead to ailments of which gout, obesity, and Bright's disease are the most common.

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[1] In addition about six grammes of hydrogen, and 700 grammes of oxygen, and thirty grammes of salts are parted with, but the dietetic value of a food depends chiefly on the amount of carbon and nitrogen it contains.

If, on the other hand, the quantity of proteid taken be kept down, so as to balance the daily loss of nitrogen, the result is that too little carbon is taken in to repair the large output, and the body consequently wastes.

Thus a purely proteid diet, though practicable for a short time, is impossible if the body is to be maintained for long in an approximately healthy condition, not to say a condition of equilibrium.

In the practical construction of a suitable diet, what is first done is to keep the quantity of proteid food at such a level as to replace the amount of nitrogen lost, and secondly to supplement this with the carbonaceous but non-nitrogenous foods, so bringing up the quantity of carbon to the requisite standard.

The non-nitrogenous foods are on this account sometimes called *proteid-sparing* foods. The same term is applied to gelatin, which within certain limits may be mixed with proteid, and so helps to supply the necessary nitrogen.

The non-nitrogenous foods are the fats and the carbohydrates; in the latter the hydrogen is already fully oxidised, and only the carbon is available for combustion; in the fats both carbon and hydrogen can undergo oxidation. There has been considerable discussion as to whether both fats and carbohydrates are essential, and as to how far one can replace the other. We shall not here enter into considerations of a theoretical nature, because the explanations they offer are not at all satisfactory; food and diet are subjects which, above all others, are intensely practical; no doubt there are satisfactory reasons for everything relating to diet, but in many cases they have still to be discovered. Practically it is found that animals thrive best on diets which supply them with the bulk of their carbon, from both fat and carbohydrate; the diets which men constructed from experience long before they had even heard of metabolism contained both fat and carbohydrate; the foods which nature has provided for growing animals, in the shape of milk and eggs, contain also both fat and carbohydrate.

Moleschott[1] fixes the following daily diet for a man performing a moderate amount of work:—

	N. in grammes	C. in grammes
120 grammes of proteid (4·232 oz. avoird.)	18·88	64·18
90 „ fat (3·174 „)	—	70·20
330 „ carbohydrate (11·64 oz. avoird.)	—	146·82
Total . . .	18·88	281·20

The total nitrogen and carbon are thus approximately equal to the total daily loss of the same two elements.

Prof. v. Ranke[2] has performed many experiments on himself, and his table of an adequate diet closely resembles Moleschott's; it is as follows:—

	N. in grammes	C. in grammes
100 grammes of proteid	15·5	53·0
100 „ fat	—	79·0
250 „ carbohydrate	—	93·0
Total	15·5	225·0

A diet consisting of fat or carbohydrate, alone or combined, is free from nitrogen, and obviously incompatible with life for more than a short period. A diet consisting chiefly of carbohydrates, as in a vegetarian diet, has disadvantages which are just the opposite as regards the carbon and nitrogen to those already fully explained in connection with a diet consisting exclusively of proteids.

Ranke and Moleschott in their experiments did not feed on proximate principles, as the above tables would seem to imply, but used the food-stuffs of every-day life, of which the percentage composition was known. Ranke's diet, for instance, consisted of meat and bread, with small quantities of potato, butter, and egg. So is it in the construction of diets nowadays. A table is consulted in which the amounts of the proximate principles in the chief foods are given (p. 573); from this the amount of such foods required to yield the necessary amount of these proximate principles can be calculated. The following tables of the ratio of nitrogen and carbon in various foods, the amount of such foods necessary for the 120 grammes of proteid, and 420 grammes of non-proteid material on the basis of Moleschott's diet will be also found useful :—[1]

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[1] *See* Pavy's *Food and Dietetics.*

[2] *Du Bois Reymond's Archiv*, 1862, p. 311, and numerous other papers.

Food	N	C	Food	N	C	Food	N	C
Beef without bone .	3	11	Oysters . .	2·1	7·2	Oatmeal . . .	1·9	44
Roast beef .	3·5	17·7	Cheshire cheese . .	4·1	41	Potatoes . . .	0·3	11
Salt cod-fish	5	16	Beans . .	4·5	42	Dried figs . .	1	34
Sardines in oil . . .	6	29	Peas . . .	3·6	44	Infusion of 3½ oz. coffee . . .	1	9
Salt herrings	3·1	23	Flour . .	1·6	38·5	Infusion of 308½ grains tea . .	0·2	2
Eggs . . .	1·9	13·5	Barley . .	1·9	40	Chocolate, 3½ oz.	1·5	58
Cow's milk .	0·6	6	Rice . . .	1·8	41	Fresh butter . .	0·6	83

Food	Weight of the food which contains	
	120 grammes of proteid	420 grammes of non-proteid (90 fat, 330 carbohydrate)
Cheese	350 grammes	1730 grammes
Lentils	453 "	693 "
Peas	537 "	704 "
Beef	566 "	1945 "
Hen's eggs . . .	893 "	776 "
Wheat bread . .	1332 "	543 "
Maize	1515 "	1751 "
Potatoes . . .	6000 "	1751 "

There is another aspect of the subject which must now be considered. The body is losing not only matter, but also energy in the shape of heat and motion.

Energy is reckoned either in the terms of work done or in equivalent heat-units.

A weight multiplied by the distance through which it is raised is a measure of work ; the units of work are called foot-tons, or foot-pounds, or metre-grammes, or metre-kilogrammes, according as the English or Continental system of weights and measures is employed.

A heat-unit, or calorie, is the amount of heat necessary to raise the temperature of 1 gramme of water 1° C. This may also be expressed in units of work, for heat is only another manifestation of energy, 'a mode of motion.'

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[1] I am indebted for the references to both these tables to Dr. McKendrick's *Physiology*. The first is from Payen, *Subst. alimentaires*, Paris, 1865; the second from Beaunis, *Physiologie humaine*, i. 627.

The following are data for calculating numbers given in one method, in terms of another :—

1 kilogramme-metre=7·233 foot-pounds ;
1 foot-pound=0·138 kilogramme-metre ;
1 kilogramme-metre=0·00328 foot ton ;
1 calorie=425·5 gramme-metres ;
=0·425 kilogramme-metre ;
1 oz. avoird.=28·35 grammes.

The loss of heat and motion is replaced by the combustion of fresh material, which ultimately comes from the food. Tables are constructed which give the heat value of food substances when burnt outside the body, and their nutritive value as a source of energy within the body is deduced from these. It must, however, be clearly understood that no combustion occurs in the food in the alimentary canal, none in the blood on its way to the tissues, but it is only after assimilation, that is, after it has become a part of the living tissues themselves, that it is oxidised and gives rise to heat and motion.

In some cases, especially that of fats and carbohydrates, the amount of heat produced when they are burnt outside the body and estimated by calorimetric processes, is the same as that produced in the interior of the body after the carbon of these substances has become the carbon of the living cells. But in other instances the *physiological heat-value* is a different thing from the physical heat-value ; this is the case with substances like proteids which are only incompletely burnt in the body. Frankland estimated that a gramme of dry proteid when burnt in the calorimeter yielded 4998 calories, or heat-units (=2124 kilo. metres of work). In the body, however, 1 gramme of proteid yields one-third of a gramme of urea. The heat-value of 1 gramme of urea is 2205 ; one-third of this (=735) deducted from 4998 gives us 4263, the physiological heat-value of 1 gramme of proteid, which, expressed in terms of work, is 1812 kilo. metres.

The energy lost *per diem* is given thus in round numbers by McKendrick.

Work of the heart	50,400	kilo. metres
Work of respiration	11,700	"
Mechanical work for 8 hours	125,000	"
Equivalent of heat produced	620,000	"
Total	807,100	
	=5,800,000	foot-pounds.

The following table is taken from Frankland,[1] and gives the force-producing ue of some important food-stuffs:—

Food	Percentage of Water	Force-producing value		
		In calories	In kilogramme-metres	
			When burnt in oxygen	When burnt in the body
Cod-liver oil . . .	—	9107	3857	3857
Beef fat	—	9069	3841	3841
Butter	—	7264	3077	3077
Cheshire cheese . .	24·0	4647	1969	1846
Oatmeal . . .	—	4004	1696	1665
Flour	—	3936	1669	1627
Pease-meal . . .	—	3936	1667	1598
Arrowroot	—	3912	1657	1657
Ground rice . . .	—	3813	1615	1591
Yolk of egg . . .	47·0	3423	1449	1400
Cane sugar . . .	—	3348	1418	1418
Hard boiled egg .	62·3	2383	1009	966
Bread crumb . . .	44·0	2231	945	910
Mackerel	70·5	1789	758	683
Lean beef	70·5	1567	664	604
Potatoes	73·0	1013	429	422
Whiting	80·0	904	383	325
White of egg . . .	86·3	671	284	244
Milk	87·0	662	280	266
Apples	82·0	660	280	273
Cabbage	88·5	434	184	178

Taking Moleschott's diet as a basis let us see how much energy is ailable from it.

Proteid 120 grammes × 1812 = 217,440 kilo. metres
Fat 90 „ × 3841 = 345,000 „
Carbohydrate 330 × 1657 = 546,810 „
Total 1,109,250 „

hich is more than sufficient to supply the energy expended; we must, wever, remember that food materials are in the first place not holly digested, in the second not completely oxidised in the body.

Variations in diet necessary in relation to work, sex, climate, c.—*Work.*—A study of prison dietaries, of military dietaries, and so rth, shows that the greater the expenditure of energy, the greater is e amount of food necessary. Muscular work falls especially on the n-nitrogenous, not on the proteids of the muscle (*see* p. 436); hence the increase of proteid food actually necessary during labour is probably smaller than in the annexed table :—[1]

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[1] *Philosophical Mag.* xxxii.

—	Ounces of					Dynamic value in foot-tons
	Proteids	Fats	Carbo-hydrates	Salts	Total	
Subsistence diet . .	2·230	0·84	11·690	—	14·760	2453
Soldiers during peace .	4·215	1·397	18·690	0·714	25·016	4026
Soldiers in the field . .	5·410	2·410	17·920	0·680	26·420	4458
Royal Engineers . .	5·080	2·910	22·220	0·930	31·140	5232
Navvy	5·640	2·340	20·410	—	28·390	4849
English sailors . . .	5·000	2·370	14·390	—	21·760	3911
Prisoner under 7 days .	1·800	0·480	10·712	—	12·992	—
" " 21 " .	2·448	0·608	14·792	—	17·848	—
" with hard labour .	4·075	1·557	18·806	1·963	26·401	4072
" with light labour .	3·508	0·315	16·727	1·715	22·265	3577
" with industrial employment . . .	3·710	1·562	17·310	1·616	24·198	3787
Prisoner with penal servitude	3·784	1·580	19·864	0·972	26·200	4193
Prisoner undergoing punishment . . .	1·296	0·256	8·160	0·368	10·080	1541

Age and sex.—Young animals require more food in proportion to their weight than adults, because they are growing in addition to maintaining metabolic processes. Aged persons require less food than those of middle age, and women less than men. The average minimum diets for different ages are thus given, in grammes :—

—	Proteid	Fat	Carbohydrate
Child under 1½ year . . .	20 to 36	30 to 45	60 to 90
" from 6 to 15 years . . .	70 to 80	37 to 50	250
Adult man (moderate work) . .	118	56	500
" woman	92	44	400
Old man	100	68	356
" woman	80	50	260

Climate.—Cold increases the appetite, increases the loss of heat, increases the desire and necessity for food with high heat-value; the fats have the highest heat-value of the proximate principles. Fat is even better than carbohydrates. Dr. McKendrick puts the matter thus: One gramme of stearin requires for its complete combustion over three grammes of oxygen, and it will only contribute 0·1 gramme of oxygen from its own substance ; while one gramme of starch requires 1·68 gramme of oxygen for its complete combustion, and it contributes about 0·5 gramme of oxygen from its own substance. Hence the combustion of stearin (as an instance of a fat) produces far more heat than the combustion of the same weight of starch (as an instance of a carbohydrate).

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[1] Taken from Sir L. Playfair, *Lecture Royal Inst.* 1865.

Digestibility of food.—In order to estimate the nutritive value of a food, it is not sufficient to calculate the percentage amount of its carbon, nitrogen, and other elements. An equally important consideration is whether the nitrogen, carbon, and other elements can be easily assimilated from that food, and since the first step towards assimilation is digestion, whether they are easily digestible.

We must, in considering this question, neglect, or almost entirely neglect, the idiosyncrasies of various people; what is easily digestible by one is difficult of digestion by another, and *vice versa;* diseases, too, especially of the alimentary canal, interfere considerably with the digestion of food. Looking, however, at the digestion of an average healthy man, the digestibility of a food depends on its source, and other extraneous circumstances, its bulk, its reaction, and the fact whether it is cooked or not.

The source of food.—The proteids of animal origin are more easily and completely digestible than those from the vegetable kingdom (*see* p. 597); the same is true for the fats; and with regard to the carbohydrates, we know that starch from some plants is more digestible than that from others. Why this should be, it is at present difficult to say. Rubner gives the following table, which illustrates some of these statements:—

—	Percentage digested of the proximate principles in									
	Meat	Eggs	Milk	Cheese	Rice	Potatoes	Peas	White bread	Black bread	Carrots
Proteid . . .	97·5	97	92	97	80	75	80	78	68	79·5
Fat	80	95	95	95	—	—	—	—	—	—
Carbohydrate	—	—	—	—	99	92·5	95	99	88	82

With regard to meat, different kinds of flesh are more easily digested than others by artificial gastric juice;[1] thus fish is more difficult to digest than mammalian meat; white flesh is more digestible than dark; raw beef than smoked. The presence of fat between the muscular fibres, as in lobster, increases the difficulty of digestion.

More valuable results have been obtained by Beaumont and Richet by observations in cases of gastric fistula; they have noted the actual time the food stayed in the stomach, and constructed tables which show that—

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[1] Chittenden and Cummins, *American Chem. Journ.* vi. 5.

a. Meat remains from $2\frac{1}{2}$ to 5 hours in the stomach, the most digestible being lamb, then in order beef, mutton, veal, pork. Fish is equal to mutton.

b. Some starchy foods (rice, barley, tapioca) remain two hours or less in the stomach; others (beans, peas, potatoes) for $2\frac{1}{2}$ hours; white bread for three, and brown bread for four hours.

The bulk of the food.—A bulky food throws excess of work on the stomach, causes discomfort, and leads to diminished absorption, as all parts of the food cannot come so well into contact with the walls of the alimentary canal. The same amount of nutriment in a more concentrated form would be more readily digested. This is one great objection to vegetarian diet. The nitrogen is so diluted by great masses of insoluble cellulose and unnecessary starch that huge volumes have to be taken to obtain the requisite minimum of fifteen grammes of nitrogen daily. The carbohydrates, too, are apt to undergo fermentative changes, and the gases so formed give rise to flatulence, and increase the discomfort.

The reaction of food.—As a rule, it is slightly alkaline; this excites a flow of gastric juice; too much alkali neutralises the gastric juice, and thus hinders digestion. Too much acid (vinegar, lemon juice, &c.) is injurious and diminishes digestion, and may lead ultimately to serious disorders of the walls of the stomach.

Cooking of food.—The cooking of foods is a development of civilisation, and much relating to this subject is a matter of education and taste rather than of physiological necessity. Cooking, however, serves many useful ends:—

1. It destroys all parasites and danger of infection. This relates not only to bacterial growths, but also to larger parasites, such as tapeworm and trichina.

2. In the case of vegetable foods, it breaks up the starch grains, bursting the cellulose, and allowing the granulose to come in contact with the digestive juices.

3. In the case of animal foods, it converts the insoluble collagen of the universally distributed connective tissues into the soluble gelatin. By thus loosening the binding material, the more important elements of the food, such as muscular fibres, are rendered accessible to the gastric and other juices. Meat before it is cooked is generally kept a certain length of time to allow *rigor mortis* to pass off. This may be hastened by hammering the flesh.

Of the two chief methods of cooking, roasting and boiling, the former is the more economical, as by its means the meat is first surrounded with a coagulated coat on its exterior, which keeps in the juices to a great extent, letting little else escape but the dripping (fat). Whereas in boiling, unless both bouillon and bouilli are used, there is considerable waste. Cooking, especially boiling, renders the proteids more insoluble than they are in the raw state,[1] but this is quite counterbalanced by the other advantages that cooking possesses.

Artificial digestion of food.—This is now a most important branch of medical practice. In patients with feeble digestions, pepsin, papain, ox-bile, and other preparations are administered to help out the insufficient supply of digestive ferments supposed to be present. It is, however, much better to digest the food for the patient before administering it; indeed, in cases where a patient has to be fed by the rectum this is almost compulsory.

Artificial gastric juice, pancreatic juice, juice of the papaw plant, &c. are employed for the purpose. The two first named are generally made from the stomach and pancreas respectively of the pig. The food, such as milk, is warmed to the body temperature, and peptonised by the addition of a certain volume of the artificial juice. The peptonisation in these artificially digested foods seldom goes further than the formation of proteoses (albumoses); true peptone is formed, if at all, in very small quantities. The best commercial preparations of peptone sold are chiefly proteoses. Artificially digested foods are usually bitter. This is a great disadvantage they have. What the bitter substance is, is unknown. It is certainly neither albumose nor peptone.

Bread-making may be considered as a partial artificial digestion, the starch of the flour being largely transformed into dextrin and dextrose.

Stutzer has elaborated the system of artificial digestion to such a pitch of excellence that, both with regard to proteids[2] and carbohydrates,[3] he is able to ascertain their digestibility with results which accord almost exactly with experiments which consist in the actual feeding of animals with the same foods, and subsequent examination of their fæces. The method of artificial digestion is by far the simpler of the two, and valuable statistics, relating chiefly to the fodder of domesticated animals, have been thus obtained. E. Pfeiffer[4] has confirmed these observations. Sheridan Lea[5] has recently attempted to imitate the conditions of normal digestion by placing the digestive mixture in a dialysing tube instead of a flask; the tube is kept constantly moving and suspended in a liquid into which the products of digestion pass out as they are formed. It was found by this means the amount digested and the rate of digestion are increased, and that intermediate products like dextrin (from starch) and anti-albumid (from proteids) are only discoverable in small quantities.

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[1] M. Popoff, *Zeit. physiol. Chem.* xiv. 524.

[2] *Zeit. physiol. Chem.* x. 301; xi. 207, 361.

[3] Stutzer and Isbert, *Ibid.* xii. 72.

[4] *Ibid.* xi. 1.

[5] *Journ. of Physiol.* xi. 226.

CHAPTER XXVIII

THE DIGESTIVE JUICES AND THEIR ACTION

Before proceeding to study in detail the character and composition of the digestive juices, and the way in which they act upon the various forms of food, it will be convenient to take a rapid survey of the whole process, and to mention the chief points in connection with each particular juice.

In the mouth mastication of the food takes place, and thorough admixture with the saliva. The saliva is an alkaline fluid containing inorganic salts and small quantities of organic matter. The two most important organic materials are *ptyalin*, an amylolytic ferment, i.e. a ferment by means of which starch is converted into sugar, and *mucin*, secreted chiefly by the submaxillary gland. The action of this slimy material is to lubricate the food, and it is in animals like the carnivora, unaccustomed to masticate their food well, or apt to swallow hard materials like bone, that mucin is most abundant, and thus the pharynx and œsophagus are protected during the process of swallowing.

The changes which the food undergoes in the mouth are chiefly mechanical, more or less fine subdivision by the teeth, partial solution and lubrication by the saliva. There is, however, a chemical change brought about by the activity of the ptyalin, a conversion of starch into sugar (maltose). This change is, however, soon cut short, for when swallowed the food enters the stomach, and there finds an acid juice ; in acid media, ptyalin is inoperative.

In some animals, however, namely, those which ingest a large quantity of starchy food, the process of insalivation is a longer one than in man. In some of these animals, such as the horse[1] and pig,[2] the secretion of acid does not begin until the food has remained some time in the stomach,[3] so that salivary digestion goes on unimpeded; in other herbivora, the ruminants, there is a still more elaborate system of insalivation which is called rumination, the food returning to the mouth after having been deposited for a time in the first compartment of the four-chambered stomach of these animals. When it returns to the mouth it is thoroughly chewed and mixed with saliva; when it is swallowed the second time it passes on to the true stomach. The ferment ptyalin in these animals is, moreover, very active, even on uncooked starch.

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[1] Ellenberger and Hofmeister, *Bied. Centr.* 1887, p. 229; Goldschmidt, *Zeit. physiol. Chem.* x. 361. [2] Ellenberger and Hofmeister, *Du Bois Reymond's Archiv*, 1888.

[3] Or it may be that the alkaline juice secreted by the stomach for the first hour or so, especially by its left half, is itself amylolytic.

The saliva acts chemically on carbohydrates only; the next juice with which the food is mixed by the peristalsis of the stomach is the gastric juice; this juice is a solution of a ferment called *pepsin*, in an acid liquid; the acid is *hydrochloric acid*. The gastric juice contains also a milk-curdling ferment. The gastric juice resembles saliva in acting on only one group of the organic proximate principles of the food. It differs from saliva in being acid, and in acting not on carbohydrates, but on proteids. The proteids are converted into the soluble and diffusible variety of proteids called peptone. This process is called proteolysis. In the case of milk, there is a preliminary curdling, due to the action of rennet. Like the conversion of starch into sugar, proteolysis is doubtless a hydrolysis (*see* p. 160, foot-note); intermediate substances between native proteids and peptones are called proteoses, of which the albumoses are the best known. The products of proteolysis are divided in many ways, but the most important subdivision is into the hemi- and the anti-groups. These names are bestowed upon proteoses and peptones according to the way in which the pancreatic juice acts upon them. The acid of the gastric juice is also valuable as an antiseptic, destroying the micro-organisms which enter with the food. Indeed, Bunge is inclined to regard this as the chief function of the gastric juice.

The next juice of importance as a digestant is the secretion of the pancreas; this enters the duodenum by an orifice which is close to that of the bile-duct. The bile, the secretion of the liver, contains special salts called bile-salts (glycocholate and taurocholate of sodium) and certain pigments related to and probably formed from hæmoglobin. The precise value of the bile as a digestant is doubtful. It appears to aid the digestion of fat, and its presence is advantageous for the due performance of the functions the pancreatic juice exercises towards carbohydrates. The bile is also stated to act as a natural purgative, and, to some extent, as an antiseptic. The pancreatic juice, however, appears to be the great digestive juice of the intestinal region. It acts upon proteids, continuing their conversion into peptones; the ferment in virtue of which it has this action is called *trypsin*; this differs from pepsin in acting only in an alkaline medium. The anti-peptones are those peptones which it is unable to decompose further; but the process of hydrolysis is carried on to the formation of leucine and tyrosine and other simpler products in the case of the hemi-peptones. The pancreatic juice possesses in addition an amylolytic ferment very like ptyalin, and a fat-splitting ferment which breaks up the fats into glycerin and fatty acids.

In the stomach the fat undergoes no change ; the proteid envelopes of the fat-cells are, however, dissolved, and thus the pancreatic juice can readily get at the fat itself. Most of the fat in the intestine undergoes the physical process of emulsification ; the globules are broken up very small, and the appearance of the contents of the intestine is milky when the diet contains fat. This finely divided fat passing into the lymphatics of the intestine gives a milky appearance to their contents (chyle), and hence their name *lacteals*.

A fourth ferment that the pancreatic juice contains is a milk-curdling ferment. It appears doubtful if this ever acts on milk during normal processes of digestion, for the milk that is acted on by the pancreatic juice has been already curdled by the rennet of the gastric juice.

The *succus entericus* is the name given to the secretion of the intestinal glands (crypts of Lieberkuhn). Much is uncertain about the action of this juice. The best ascertained fact in respect to it seems to be the presence of an inverting ferment (*invertin*) which transforms sucroses, such as cane sugar and maltose, into dextrose.

Another process which occurs in the intestine is the activity of bacterial ferments. It is sometimes difficult to say where pancreatic digestion ends and putrefaction begins, since many of the products of both actions (leucine, tyrosine, phenol, &c.) are the same. Gases are produced from both carbohydrates and proteids ; free fatty acids and lactic acid are formed. Indole and skatole are produced from the proteids, and give the contents of the intestine their characteristic odour.

Such, then, is a *résumé* of the chief chemical activities occurring in the alimentary canal. Summing up the subject from another standpoint, we have the following :—

1. The carbohydrates are acted upon by—

(*a*) The saliva—starch changed into maltose.

(*b*) The pancreatic juice—starch changed into maltose.

(*c*) The succus entericus—cane sugar and maltose changed into dextrose.

2. The fats are acted upon by—

(*a*) The pancreatic juice.

(*b*) The bile.

The net effect is chiefly to break up fat into minutely subdivided particles (emulsification); partly to break it up into glycerin and fatty acids; the latter combining with alkaline bases form soaps (saponification).

3. The proteids are acted upon by—

(*a*) The gastric juice.

(*b*) The pancreatic juice.

Proteoses and peptones are formed. Some peptones are further broken up by trypsin into leucine, tyrosine, &c.

4. All three varieties of the organic proximate principles are acted upon to some extent by the putrefactive organisms of the intestines. These no doubt fulfil a useful purpose, but are probably kept in check by the natural antiseptic, the bile.

5. The water and mineral salts undergo no change.

The object of digestion is to form substances which will be easily absorbed, that is, pass readily by processes of diffusion, filtration, &c. into the blood and lymph in the vessels of the stomach and intestines. Thus we get sugars formed from the colloid starch, peptones from the non-diffusible proteids. The absorption of the emulsified fats is a question which we shall see is fraught with difficulties.

A certain proportion of the food ingested is, however, not digested. Cellulose in vegetable tissues, keratin and elastin in animal tissues are instances of chemical materials, but little affected by digestive processes. These and others of the same sort, together with débris from the intestinal wall, residues from the bile and other secretions, and products of putrefaction constitute the excrementitious matters, or fæces, which are got rid of *per rectum*.

CHAPTER XXIX

SALIVA

The saliva is the first digestive juice which comes in contact with the food ; it is secreted by three pairs of salivary glands, the parotid, the submaxillary, and the sublingual. The saliva secreted by the different glands varies somewhat in composition ; the secretion enters the mouth by means of the ducts of the glands ; it there becomes mixed ; the secretion of the minute mucous glands of the mouth and a certain number of epithelial cells and débris are also added to it. The subject may be conveniently considered under the four following heads :—

1. The physiology of salivary secretion.
2. The structure of the cells that secrete saliva.
3. The composition of saliva.
4. The action of saliva on foods.

1. THE PHYSIOLOGY OF SALIVARY SECRETION

The secretion of saliva is a reflex action ; under normal circumstances the mouth is moistened with saliva, but there is no excess of secretion present. When food is taken, or any substance is placed in the mouth, a copious flow is produced. An impulse set up in an afferent nerve reaches the nerve-centre which controls salivary secretion ; an impulse thence travels along an efferent nerve to the gland, and there influences the cells and causes them to secrete abundantly. The sight of food, or its smell, or certain conditions of the stomach (as in nausea) may also reflexly cause salivary secretion.

The efferent nerves just alluded to contain certain special secreting fibres. In the early days, when the existence of secretory nerves was first mooted, objectors advanced the counter-theory, that the effects observed were due to the action of the vaso-motor nerves, which by affecting the size of the blood-vessels produced differences in the amount of blood supplied to the gland, and thus caused a difference in the amount and rate of secretion. In support of this they showed that the *chorda tympani*, a branch of the seventh cranial nerve which supplies the submaxillary gland, contains numerous vaso-dilatator fibres; when this nerve is stimulated there is an increased redness of the gland, owing to the dilatation of its blood-vessels, and there is also an increase in the flow of saliva. Further observation soon showed, however, that the increased flow of secretion is not due to the increased vascularity of the secreting organ, for the three following reasons:—

a. The pressure in the duct is often higher, sometimes twice as high as the pressure in the arteries.

b. If the experiment be performed on the head of a recently decapitated rabbit, stimulation of the nerve still produces a flow of saliva; and here there cannot be any interference from alterations of blood-pressure.

c. By the use of small doses of the alkaloid atropine, the two kinds of fibres contained in the nerve can be differentiated one from the other; the drug produces paralysis of the secretory fibres, but it has no effect on the vaso-dilatator fibres; excitation of the nerve produces under these circumstances a dilatation of the blood-vessels of the gland, but no increased flow of saliva.

The next question which arises is this, Admitting the existence of secretory nerves, is there any histological evidence that nerve-fibres terminate in secretory cells? The answer to this is an unsatisfactory one; the nerve terminations are probably connected with the cells, but the exact method of connection has not at present been ascertained. Pflüger made observations in which he described the direct connection of nerve-fibres with the nuclei of the salivary cells, but his assertions have never been corroborated.

The submaxillary gland is the salivary gland in which the nervous mechanism of secretion has been most fully worked out. We will therefore consider this gland first. It is supplied by two nerves, the chorda tympani, and by branches of the cervical sympathetic, which enter the gland with its artery, and supplies vaso-constrictor fibres to it. We have already seen that the chorda tympani supplies the gland with secretory fibres, and its vessels with vaso-dilatator fibres; the sympathetic supplies the vessels with vaso-constrictor fibres. Has it any secretory fibres? Such a question can only be answered by means of experiment—the experiment of stimulating the cervical sympathetic nerve. When this is done not only are the vessels constricted, but in the dog a slight flow of saliva results, which is remarkably viscid, of higher specific gravity and richer in corpuscles than is the chorda saliva. In different animals the results varies; thus in the rabbit, both chorda and sympathetic saliva are free from mucin, but the latter contains more proteids; in the cat, chorda saliva is more viscid than sympathetic saliva ; but in all these animals the sympathetic saliva is smaller in quantity than the chorda saliva, and in all of them the blood-vessels are constricted.

To explain this difference between the action of the two nerves of the gland, Heidenhain[1] has advanced the theory that the cells of a secreting gland are supplied by two kinds of nerves ; the one, *trophic*, exciting chemical processes in their protoplasm ; the other, *secretory*, having to do with the separation of the secreted products. In all cells, gland-cells among the number, two processes are continually occurring: one the building up of their substance and contents (anabolism), the other the breaking down of the same (katabolism). That each of these processes is governed by a special nerve-filament was an ingenious speculation, which it turns out, on further investigation, is supported in several ways. The existence of the two kinds of fibres, and their admixture in various proportions with one another, and with vaso-motor fibres, will explain very largely the result of stimulation of the nerves we have mentioned ; to take the case of the dog's submaxillary again, the chorda contains many secretory fibres and few trophic fibres ; hence the secretion which follows its stimulation is copious and watery. The sympathetic, on the other hand, contains few secretory and many trophic fibres ; hence the secretion which follows its stimulation is scanty and viscid.

Bayliss and Bradford[2] have confirmed the probable existence of Heidenhain's two sets of fibres by demonstrating that the electrical changes in the glands are of the opposite kind on stimulation of the two nerves ; and that atropine destroys the chorda variation (hilus positive to surface of gland), but only slightly lessens the sympathetic variation (hilus negative to surface).

Langley, however, considers that the existence of more than one kind of secretory fibre is very doubtful ; and he shows, too, that this assertion is not irreconcilable with the conclusions of Bayliss and Bradford. The reasons for Langley's conclusions are entered into fully in the papers quoted below,[3] and briefly they are these :—

(1) The phenomena of atropine-poisoning give no indication of the existence of more than one kind of secretory nerve-fibre. By the use of very small doses of atropine, administered successively, all varieties of secretory nerve-fibre are equally and simultaneously paralysed.

(2) Experiments on the submaxillary, in which the two nerves supplying the gland are alternately stimulated, also tend to throw doubt on the existence of two varieties of nerve-fibre. The sym-

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[1] Hermann's *Handbuch*, 1880, vol. v.

[2] *Proc. Roy. Soc.* xl. 203.

[3] *Journ. Physiology*, ix. 55; x. 291.

pathetic saliva is largely increased in amount by previous stimulation of the chorda, that is, after the increased supply of blood produced by dilatation of the blood-vessels. Unless the gland has been thus previously supplied richly with oxygen, the secretory fibres of the sympathetic (which are comparatively few in number and masked by admixture with vaso-constrictor nerves) are non-effective or nearly so. Langley thus considers that the action of the nerve-fibres on the size of the vessels has more importance than Heidenhain was inclined to give to it; and that the secretory fibres being in the two nerves mixed with vaso-motor fibres of opposite kinds, explains the difference in the actions of the nerves quite as well as or better than the hypothesis that the secretory fibres are themselves of opposite kinds.

Whichever explanation is ultimately shown to be correct—and there is much to be said on both sides—there is little doubt that the parotid and the sublingual are governed by nervous influences in the same way as is the submaxillary gland. Stimulation of the sympathetic in the dog produces no secretion of saliva from the parotid gland, or only when the gland has been previously thrown into a state of increased irritability by the previous stimulation of a nerve which corresponds to the chorda tympani in relation to the submaxillary: this nerve is a branch of the glosso-pharyngeal nerve called Jacobson's nerve, which may be reached within the tympanum, in the tympanic plexus.

Paralytic secretion.—This is a thin, watery secretion that occurs about twenty-four hours after section of the secretory nerve. The gland of the opposite side is also affected (antilytic secretion; Langley). It begins to diminish about the eighth day. It has been explained as a degeneration effect comparable to the fibrillar contraction of muscle, and also as caused by the action of venous blood, increasing the excitability of local centres in the gland.

2. THE STRUCTURE OF THE CELLS THAT SECRETE SALIVA

As much as is known concerning the chemical constituents of the salivary glands *en masse* has been already referred to (p. 558). Microscopical examination of the cells of the glands in relation to the time and amount of secretion teaches us certain important facts concerning the elaboration of the important substances ptyalin and mucin.

The first fact of importance noted in sections from hardened specimens is that certain cells are filled with a highly refracting substance called mucinogen, which is subsequently extruded as mucin when these cells degenerate; they press the more protoplasmic and more easily stainable cells to the basement membrane, where they form the so-called crescents of Gianuzzi. Cells of this nature are called mucous cells ; the alveoli in which they occur, mucous alveoli or acini ; the glands which contain mucous alveoli are called mucous glands ; these are in man the submaxillary and the sublingual ; the secretion of the mucous glands is called mucous saliva. There are other cells arranged also in acini, which according to their state of secretory activity are more or less filled with fine granules ; the granules, however, will be invisible in a section of the hardened organ ; there are no crescents ; these are termed serous cells, serous acini, serous glands respectively. Such a gland is the parotid. Some acini of the submaxillary and sublingual are also serous. The saliva secreted by these is called serous saliva. The saliva is in other words limpid and not viscid. The use of the word serous in this connection is established by long usage, but is singularly unfortunate ; the parotid saliva and the serum are alike in that mucin is absent from both, but here the resemblance ends. Foster [1] suggests the word *albuminous* instead.

The changes in the cells and their structure can be made out better by teasing fresh preparations of the gland in serum, or aqueous humour, or after exposure to weak osmic acid, or better still osmic acid vapour (Langley) ; this reagent preserves the granules, and the preparations can be subsequently stained with carmine, hæmatoxylin, &c., and kept.

When we study an albuminous gland in the fresh, living condition, the changes during activity are like those already described in more general terms in connection with secreting epithelium. In the stage represented in A (fig. 82) the cells are large, their outlines very indistinct, and the cell-substance studded with minute granules. This stage is generally called the stage of rest ; rest is here a comparative term. The building up of these granules within the protoplasm is really a stage of activity, but a different kind of activity to that which follows. These granules are composed of or indicate the presence of the precursor of the ferment. Ferment precursors are also

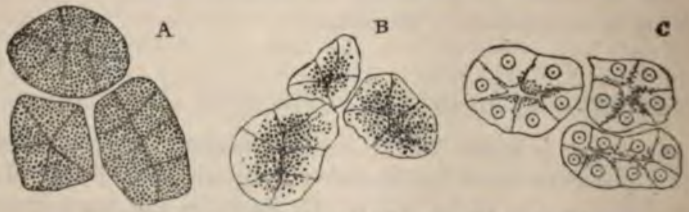

FIG. 82.—Alveoli of Serous Gland in different conditions of activity.

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[1] Foster's *Physiology*, 5th edition, ii. 406.

called zymogens; and this particular zymogen may be called *ptyalinogen*, the precursor of ptyalin. The stages which follow (fig. B and C) are usually spoken of as stages of activity; they are more correctly the stages seen while secretion is taking place, or after it has occurred. The cells become smaller (B) as they shed out the secretion, their outlines and nuclei more distinct, and the granules disappear, especially from the outer parts of each cell. After prolonged activity (C) such as is produced by the injection of pilocarpine, or by stimulation of the secretory nerve, these changes are all more marked; only a few granules are left at the free border of the cells, which now abut on a conspicuous lumen.

These granules are dissolved or rendered indistinct by alcohol, chromic acid, and other hardening reagents.

In a mucous gland the changes that take place are of a like kind.[1] If a piece of resting, or, to speak more correctly, loaded submaxillary gland be teased out, spherules are seen which are larger than the granules of the parotid and less dense and solid than those of the pancreas. These crowd the cell-protoplasm (fig. 83, *a*). In a discharged gland, that is, one which has been secreting for some time, the granules are less numerous and largely confined to the part of the cell near the lumen (fig. 83, *b*). The distinction between an inner 'granular zone' and an outer 'clear zone' next to the basement membrane is less distinct than in the serous or albuminous acini, partly because the granules do not disappear in so regular a manner as in the parotid and pancreas, and partly because the outer zone of the mucous cell is less homogeneous.

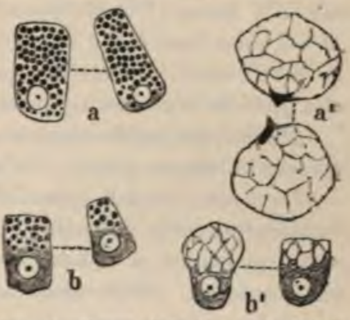

FIG. 83.—Mucous Cells from a fresh Submaxillary Gland of Dog (from Foster, after Langley).

In fig. 83, *a'* and *b'* represent cells in a loaded and discharged condition respectively, which have been irrigated with water or dilute acid. The mucous granules (mucinogen) are swollen into a transparent mass of mucin traversed by a network of protoplasmic cell-substance; the appearance of mucous cells in sections hardened by alcohol and stained in the usual way is very similar.

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[1] Langley, *Phil. Trans.* 1889.

3. THE COMPOSITION OF SALIVA

Mixed saliva.—The saliva as found in the mouth is a mixture of that from all the different glands. On microscopic examination, a few epithelial scales from the mouth and salivary corpuscles from the salivary glands are seen. The liquid is transparent, slightly opalescent, of slimy consistency, and may contain lumps of nearly pure mucin. On standing, it becomes more cloudy, owing to the precipitation of calcium carbonate, the carbonic acid which held it in solution as bicarbonate escaping.

Its constituents are:—

Organic.—(*a*) Mucin. Acetic acid precipitates this in a stringy form.

(*b*) Ptyalin: an amylolytic ferment discovered by Leuchs[1] in 1831. It is constantly present in human saliva, even in new-born children;[2] it is usually absent in dog's saliva;[3] it was not found by Roux in the saliva of horses;[4] Schiff found it in that of rabbits and guinea-pigs.[5] It has since been found in nearly all animals.

(*c*) Proteid. A trace of a proteid, coagulable by heat, of the nature of a globulin is constantly present.

(*d*) Sulphocyanide of potassium (KSCN) is usually, but not always, present in human saliva.[6] It is absent in dog's saliva.[7]

Inorganic.—Small quantities of chlorine and phosphoric acid in combination with potassium, sodium, calcium, and magnesium; also small quantities of sodium carbonate. Sodium chloride is the most abundant salt. Schönbein,[8] observed that saliva contains a substance which, like nitrous acid, colours blue a mixture of starch and potassium iodide. The nature of this substance is doubtful.

Impurities.—If putrefactive processes be taking place in the mouth, bacteria will be found; indeed, they are never altogether absent. The saliva may under these circumstances be acid. It is also acid in some cases of diabetes mellitus; sugar, is however, always absent; and so is bile in cases of jaundice. Certain drugs, especially iodine, appear quickly in the saliva after they have been administered. In fevers the amount of saliva secreted is much lessened. Tartar consists chiefly of calcium phosphate and carbonate, admixed with mucus and leptothrix.[1] The so-called 'tooth-stones' have the same composition.

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[1] Kastner's *Arch.* 1831. *See* also Schwann, *Pogg. Ann.* xxx. 358.

[2] Schiffer, *Arch. f. Anat. und Physiol.* 1872, p. 469; Korowin, *Centralbl. med. Wiss.* 1873, No. 17.

[3] Hoppe-Seyler, *Physiol. Chem.* p. 186.

[4] *Gazz. med. veterin. de Milano,* 1871.

[5] Schiff, *Leçons sur la physiol. de la digestion,* 1868.

[6] Treviranus, *Biologie,* vol. iv. 1814, p. 330; Tiedemann and Gmelin, *Die Verdauung nach Versuchen,* vol. i. 1826, p. 9.

[7] Hoppe-Seyler, *Physiol. Chem.* p. 186.

[8] *Journ. prakt. Chem.* lxxxvi. 151. *See* also Schaer, *Zeit. Biol.* vi. 467.

Quantitative analysis.—The quantity of saliva secreted daily by a man varies considerably; estimates varying between 13 oz. and 3½ lb. have been given; 500 to 800 grammes is another estimate—oxen and horses may secrete 40,000 to 60,000 grammes daily. Its alkalinity averages in man ·08 per cent. expressed as sodium carbonate (Chittenden).

Its specific gravity is 1002 to 1006 in man; 1007 in dogs. It contains in man five parts of solid matter per 1000, of which two are inorganic.

—	Human mixed saliva			Dog's mixed saliva[3]
	I[2]	II[3]	III[4]	
Water	994·10	995·16	994·7	989·63
Solids	5·90	4·84	5·3	10·36
Soluble organic matter	1·42	1·34	3·2	3·57
Epithelium	2·13	1·62	—	—
Sulphocyanide of potassium	0·10	0·06	—	—
Salts	2·19	1·82	1·03	6·75

Submaxillary saliva.—A cannula is inserted into Wharton's duct, and the saliva obtained by the stimulation either of the chorda tympani or sympathetic can be readily collected and examined.

The saliva thus obtained is colourless, clear, transparent, and sticky, especially if obtained by stimulation of the sympathetic. It is markedly alkaline, and soon becomes cloudy in the air from deposition of calcium carbonate.

Its composition is in the main the same as that of mixed saliva; the mucin is more abundant; the proteid coagulable by heat is not always present. Ptyalin is present in human submaxillary saliva, except in infants under the age of two months (Zweifel). It is present in most animals, but not in dogs. Potassium sulphocyanide is present in human, but not in dogs' submaxillary saliva.[5] The inorganic salts are calcium carbonate, calcium and magnesium phosphate, potassium and sodium chloride.[6]

Quantitative analysis (in parts per 1000 dog's submaxillary saliva):—

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[1] Vergne, 'Du tartre dentaire et de ses concrétions,' *Thèse*, Paris, 1869.

[2] Frerichs, Wagner's *Handwörterbuch d. Physiol.* iii. 758.

[3] C. Schmidt and Jacubowitsch, *Ann. Chem. Pharm.* lxxix. 156.

[4] Herter, Hoppe-Seyler's *Physiol. Chem.* p. 188.

[5] Zweifel, *Untersuchungen ü. d. Verdauungsapparat. d. Neugeb.* Strasburg, 1874.

[6] Longet, *Compt. rend.* xlii. 480; Oehl, *La saliva umana*, Pavia, 1864.

—	Bidder and Schmidt[1]		Herter[2]			
	I	II	III	IV	V	VI
Water	996·04	991·45	994·385	994·969	995·411	991·319
Solids	3·96	8·55	5·615	5·031	4·589	8·681
Organic matters	1·51	2·89	1·755	—	—	—
Mucin[3]	—	—	0·662	—	—	2·604
Salts	2·45	5·66	3·870	—	—	7·332
CO_2 in chemical union	—	—	0·440	0·504	0·654	—

Salts[4] (dog)	
K_2SO_4	0·209 per 1000
KCl	0·940 „
NaCl	1·546 „
Na_2CO_3	0·902 „
$CaCO_3$	0·150 „
$Ca_3(PO_4)_2$	0·113 „

Gases[5] (dog)	
Oxygen	0·4 to 0·6 vols. per cent.
CO_2 (free)	19·3 „ 22·5 „
O_2(combined)	29·9 „ 42·2 „
CNitrogen	0·7 „ 0·8 „

The main facts concerning the difference in the secretion produced by excitation of the two nerves of the gland have been already mentioned (p. 617). The following are actual analyses (given in percentages) of the saliva thus obtained :—

I. By stimulation of the chorda tympani.[6]

	Organic matters	Ash	Total solids
a. Weak stimulation	1·5987	0·519	2·1187
b. Strong „	2·5047	0·629	3·1339

A strong stimulus thus produces an increase in the total solids, especially of the organic solids, and particularly of the mucin. That the percentage of salts in the saliva also increases with the rate of secretion was also noted by Werther,[7] who obtained as high a percentage in some cases as 0·77. Langley and Fletcher[8] have more recently obtained the same results, both by means of stimulating the chorda, or injecting small doses of pilocarpine, a drug which increases the rate of salivary secretion.[9] Such a fact goes to prove that the secretion of even water and salts is an act of the secreting cell, and not simply due to increased transudation from the blood.

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[1] Bidder and Schmidt, *Ann. Chem. Pharm.* vol. lxxix.

[2] Hoppe-Seyler's *Physiol. Chem.* p. 191.

[3] In rabbits' submaxillary saliva, mucin is absent.

[4] Herter, *Loc. cit.*

[5] Pflüger, in Heidenhain's *Studien des Physiol. Inst. Breslau*, Leipzig, Heft iv. p.

[6] Heidenhain, *Ibid.*

[7] *Pflüger's Archiv*, xxxviii. 29

[8] *Phil. Trans.* 1889, vol. clxxx. B, p. 109.

[9] A very complete account of the antagonistic action of atropine and pilocarpine on salivary secretion will be found in *Journ. Physiol.* i. 339 (Langley). Another important contribution on the influence of nicotine on salivary secretion, by the same author, will be found in *Journ. Physiol.* xi. 123.

II. By stimulation of the sympathetic (dog).

Here we get a small quantity of saliva, which is richer in solids than chorda saliva, especially in mucin and formed elements. Heidenhain, moreover, found that the percentage of solids falls after prolonged stimulation; thus :—

	Amount of secretion	Percentage of solids
In the first 80 minutes	0·6774 gramme	3·744
In 88 minutes, after stimulation had lasted 190 minutes	0·8871 „	1·488
In the first 40 minutes	0·5286 „	5·864
In 30 minutes, after stimulation had lasted 80 minutes	0·5330 „	1·910

Sublingual saliva.—The secretion of the sublingual gland does not materially differ from that of the submaxillary. It is, however, the richest of the salivas in solids (2·75 per cent. Heidenhain), formed elements, mucin, and inorganic salts; it is thus the most viscid and the most alkaline (Heidenhain, Werther, Langley).

In certain birds this gland is much enlarged, and secretes the viscid material out of which they build their nests (*see* Neossin, edible bird's-nest, p. 486).

Parotid saliva.—The parotid gland yields a watery secretion, free from mucin, and rich in ptyalin, even in the new-born child. The gland is more highly developed in vegetable feeders than in carnivora. The saliva can be easily collected, especially in dogs, by means of a cannula in Stenson's duct. Its characters, with the exception of sliminess, and its constituents, with the exception of mucin, are the same as in submaxillary saliva. It always contains a small quantity of a globulin.

Quantitative analysis.—On the next page are some analyses in parts per 1000. The table is compiled by Hoppe-Seyler.[1]

The Secretion of the Mucous Membrane of the Mouth

When the ducts of all the salivary glands have been ligatured a small quantity of very viscid secretion is poured into the mouth by the mucous glands of its lining membrane. Jacubowitsch gives the following analysis of this secretion obtained from a dog :—

Solids	9·98 per 1000
Organic solids	3·85 „
Inorganic solids	6·13 „

It has no diastatic action. The mucus secreted by the tongue of the frog (an animal with no salivary glands) is, however, diastatic.

The *poison-glands* of snakes are modified salivary glands. The secretion is

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[1] *Physiol. Chem.* p. 199.

Constituents	Human parotid saliva		Dog's parotid saliva				Horse's parotid saliva.
	I Mitscherlich	II Hoppe-Seyler	III Schmidt and Jacubowitsch	IV	V Herter	VI	VII Lehmann
Water	983·7 to 985·4	993·16	995·3	993·85	991·527	991·928	[illegible]
Solids	14·6 to 16·3	6·84	4·7	6·15	8·473	8·072	[illegible]
Organic matters	9·0	3·44	1·4	—	1·536	—	2·06 to [illegible]
KSCN	0·3	—		—		—	—
KCl	5·0	3·40	2·1	—	6·251	—	4·8 to [illegible]
NaCl				—		—	
$CaCO_3$			1·2	—	0·688	—	
Specific gravity	1006 to 1008		1004 to 1007				1005 to [illegible]
Gases[1]	100 c.c. of saliva yielded 7 c.c. of gas (1 c.c. oxygen, 2·5 nitrogen, and 3·5 carbonic anhydride). By adding phosphoric acid 40 to 60 c.c. of carbonic anhydride were obtained.						

rich in proteids, and the poison is a proteid one (*see* Proteids as Poisons, p. 137). The specific gravity of snake poison is over 1040. Its reaction is in some cases alkaline, in others weakly acid; it is usually described as yellowish and viscid.

4. THE ACTION OF SALIVA

The active principle of saliva is ptyalin. This belongs to the class of unorganised ferments, that are called either amylolytic (starch-splitting), or diastatic (resembling diastase, the similar ferment in germinating barley and other grains).

Ptyalin may be prepared from a watery infusion of a minced salivary gland or from the saliva itself. Dilute phosphoric acid is added, and this is neutralised with lime-water; the precipitate of calcium phosphate which is formed carries down the ptyalin with it; this is collected on a filter and water added; the water dissolves out the ptyalin, leaving the phosphate on the filter. The ptyalin is then precipitated from its aqueous solution by adding excess of alcohol. The precipitate may be collected, dried, and preserved for future use. It may be purified by re-dissolving in water, and again precipitating with alcohol. To obtain a glycerine extract, a minced salivary gland is covered with absolute alcohol for twenty-four hours; the gland substance freed from alcohol is dried, powdered, and allowed to macerate in strong glycerine for several days; the ptyalin may then be precipitated from the glycerine solution by alcohol as before.

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[1] R. Kulz, *Zeit. Biol.* xxiii. 321.

The only important chemical action of saliva is that due to the presence of ptyalin. It has various physical actions; it dissolves certain substances, enabling us to taste them; in virtue of its mucin, it lubricates the bolus before it is swallowed; in virtue of its viscidity and alkalinity, it has a feeble, emulsifying action on fats.

The diastatic activity of saliva may be readily demonstrated by the following simple experiment :—

A few cubic centimetres of starch solution are placed in a test-tube, and a few drops of saliva added; the tube is placed in a warm bath at 35° C. and by means of a glass rod a drop is removed every half-minute, and mixed with a drop of dilute solution of iodine on a testing slab. At first the drop strikes a deep blue from the presence of starch; after a few minutes, another drop gives a violet colour; this is because the starch is gradually disappearing, but some is still left, and the violet colour is produced by admixture of the blue tint, due to starch, and the reddish tint, due to dextrin into which the starch is being converted; in a few minutes more a fresh drop strikes a reddish brown with iodine, showing that all the starch has disappeared; and in a few minutes more, a fresh drop gives no colour at all with iodine, showing that the dextrin which gave the red colour has also gone. If at this stage a little of the fluid be withdrawn and alcohol added in excess, a white precipitate is produced; this cannot be starch, as all the starch has long ago disappeared; it cannot be sugar, as sugar gives no precipitate with alcohol; it cannot be the dextrin that gave the red colour with iodine, as there is no longer a red colour given with iodine; if analysed, however, it is found to have the same composition as dextrin, and thus it is called *achroo-dextrin*; while the dextrin which gave the red tint is called *erythro-dextrin*. If we test the liquid at the various stages by means of Trommer's test or Fehling's solution for sugar (p. 95), we shall find sugar present as soon as dextrin appears; it increases as the dextrin disappears. Achroo-dextrin is, however, only partially, and with great difficulty, converted into sugar. This simple experiment teaches us that starch is transformed into dextrin and sugar, and that ultimately the greater part of the dextrin is also changed into sugar.

Nasse[1] was the first to show that this sugar is not dextrose, and called it *ptyalose*. v. Mering and Musculus[2] conclusively proved that ptyalose and maltose (the sugar formed by diastase in malting) are identical.

We have already in our consideration of the carbohydrates seen how this transformation may be represented by a chemical equation; the equations given by various authors differ according to the view they take of the molecular constitution of starch and the dextrins.

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[1] *Pflüger's Archiv*, xiv. 473.

[2] *See* Seegen's paper, *Pflüger's Archiv*, xl. 38.

The formula given by Brown and Morris is probably more correct than most of the others; it is:—

$$\underset{[\text{starch}]}{10(C_6H_{10}O_5)_n} + \underset{[\text{water}]}{4nH_2O} = \underset{[\text{maltose}]}{4nC_{12}H_{22}O_{11}} + \underset{[\text{achroo-dextrin}]}{(C_6H_{10}O_5)_n} + \underset{[\text{erythro-dextrin}]}{(C_6H_{10}O_5)_n}$$

The chief properties of maltose have been already described (p. 103). Small quantities of lactic acid are formed at the same time.[1]

Ptyalin acts in a similar way, but more slowly on glycogen; it has practically no action on cellulose; hence it is inoperative on uncooked starch grains, in which the cellulose layers are intact.

Ptyalin acts best at about the temperature of the body (35° to 40° C.); diastase acts most energetically at 60° C.[2]

Ptyalin acts best in a neutral medium; a small amount of alkali makes but little difference; a very small amount of acid stops its activity; hence the action of the saliva stops when the food reaches the stomach containing acid gastric juice. The gastric juice not only stops its action, but destroys the ferment, so that it does not resume work when the semi-digested food becomes once more alkaline in the duodenum.[3]

In the human subject and in certain other animals v. d. Velden[4] showed that there is no free acid in the stomach until about three-quarters of an hour after the arrival of the food there. It was therefore supposed that during this time, the ptyalin was able to exert its activity. This hypothesis was confirmed by the observations of Chittenden and Ely, which showed that saliva neutralised with acid is more active than ordinary alkaline saliva; the acid first secreted by the stomach is thus presumably used in the neutralisation of saliva, and is consequently an acid to the already powerful ferment, ptyalin, of that secretion. The subsequent experiments of Langley and Eves showed, however, that this is not altogether correct, for in the stomach, when an ordinary mixed diet is being used, proteids are present, and acid proteids or acid peptones have a distinctly retarding action on ptyalin. In all probability, therefore, the conversion of starch into sugar by ptyalin in the stomach stops after fifteen to thirty minutes, that is before any free acid appears. Free hydrochloric acid immediately destroys the ptyalin.

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[1] Goldschmidt, *Zeit. physiol. Chem.* x. 273.

[2] *See* Stutzer and Isbert, *Zeit. physiol. Chem.* xii. 72.

[3] J. N. Langley, *Journ. Physiol.* iii. 246; Langley and Eves, *Ibid.* iv. 18; Chittenden and Ely, *Ibid.* iii. 327; Chittenden and Smith, *Chem. News*, vol. liii. (six contributions).

[4] *Zeit. physiol. Chem.* iii. 205.

The following is a *résumé* of the work of Chittenden and Smith on the subject of the influence of reaction on the activity of ptyalin. For the purpose of testing this action quantitatively a known amount of a 1 or 2 per cent. solution of starch was exposed to the action of a measured quantity of saliva at 40° C. for thirty minutes; it was then boiled to stop further action, and the sugar in it estimated. The action of a ferment is not proportional to its amount until its solution is much diluted; when the dilution of saliva is as 1 : 50 or 100, the diastatic action can be taken as a measure of the amount of ferment present. The normal alkalinity of fifteen samples of saliva reckoned in terms of sodium carbonate was 0·097 per cent. When this is neutralised with 0·2 per cent. hydrochloric acid its diastatic action is much increased, especially when the dilution is 1 to 50 or 100, but the difference is still pronounced when the dilution reaches 1 to 2000. There appears to be no proportional relation between natural variations of alkalinity and diastatic action, although the addition of sodium carbonate to neutral saliva retards and finally stops the action of ptyalin in proportion to the amount added. This occurs especially readily in more dilute solutions; this is not due to simple dilution, but to the thereby diminished percentage of proteid matter, which in the less diluted saliva possibly combines with the carbonate, such proteid compounds having no effect on the ferment. Neutral peptone, on the contrary, has a distinctly stimulating effect on the activity of neutral saliva; and when proportionate amounts of peptone and sodium carbonate are added the distinctive action of the latter is prevented, an alkaline proteid compound being probably formed.

These investigators then proceeded to determine quantitatively the amount of acidity necessary to stop diastatic action, the tropæolin test being used for the detection of free acid.[1] As a mean of eight determinations, 20 c.c. of neutralised saliva were found to contain proteids capable of combining with 7·74 c.c. of a 0·1 per cent. solution of hydrochloric acid. When the proteid matter present is saturated with acid the saliva has greater diastatic power than when simply neutralised. Small percentages of acid peptone act similarly, but beyond a certain point (when the amount of combined acid is over 0·006 per cent.) acid proteids retard and finally destroy the action of the ferment. A minute trace of free acid in dilute saliva still further increases its diastatic activity; this trace is, however, so minute as to be for practical purposes inappreciable, for 0·003 per cent. of free hydrochloric acid entirely stops the diastatic activity of saliva.

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[1] Drops of a saturated solution of tropæolin 00 in 94 per cent. methylated spirit, are allowed to dry on a porcelain slab at 40° C. A drop of the fluid to be tested is placed on the tropæolin drop, still at 40° C., and if hydrochloric acid is present a violet spot is left when the fluid has evaporated. A drop of HCl 0·006 per cent. thus leaves a distinct mark. (Danilewsky, *Centralbl. med. Wiss.* 1880; Szabo, *Maly's Jahresb.* vii. 267; v. d. Velden, *Ibid.* x. 305.)

CHAPTER XXX

GASTRIC JUICE

THE juice secreted by the glands in the mucous membrane of the stomach varies somewhat in composition in the different regions, but the mixed gastric juice, as it may be termed, is a solution of a proteolytic ferment called pepsin in a saline solution, which also contains a little free hydrochloric acid. We find, as in the case of saliva, variations in the importance of the gastric juice in different parts of the animal kingdom, the most powerful juice being that obtained from carnivorous animals, whose diet is almost exclusively proteid. The gastric juice of new-born children is quite active (Zweifel).

The saliva is a juice which is readily obtainable. The gastric juice, on the other hand, cannot be reached until the animal is either killed, or an operation, that of making a gastric fistula, performed. In man gastric fistulæ are also necessary in cases of disease; for instance, a tumour occluding the œsophagus would kill its possessor by starvation, unless the stomach were opened, stitched to the wall of the abdomen, and food introduced through the artificial opening. From such cases gastric juice is obtainable, but the result of examining the juice is not absolutely satisfactory. We cannot be sure that it has a normal character if the person from whom it is removed is, as is usually the case, in a depressed state of health.

The most celebrated case of gastric fistula, and the first upon which trustworthy observations were made, is that of Alexis St. Martin, a young Canadian who received a musket wound in the abdomen in 1822. He fell into the hands of Dr. Beaumont, who not only saved his patient's life, but took him into his service and then conducted a series of important experiments on him. He was able to collect the juice and to observe the vascular and other conditions of the stomach during digestion of different foods, during rest, and in minor derangements of the alimentary canal. The perforation in the stomach, though ordinarily closed by a loose flap of mucous membrane, was 2½ inches broad, thus enabling the painstaking observer ample scope for his investigations

Dr. Beaumont's discoveries are described by him in his book on the 'Physiology of Digestion.'[1] Since his time somewhat similar cases have been carefully observed by Richet,[2] Grünewaldt,[3] Schröder,[4] and others.

The amount of gastric juice secreted daily is differently estimated by various observers. Beaumont by mechanically stimulating the gastric mucous membrane obtained on the average $1\frac{1}{2}$ fluid ounce : reckoning three meals a day this would give a daily secretion of four to five ounces (135 to 180 grammes). Bidder and Schmidt in dogs obtained about forty times that quantity, and Grünewaldt in his case of human gastric fistula gives a daily mean of 580 grammes.

The description of the properties and action of the gastric juice may be now conveniently taken under the four following heads :—

(1) The physiology of the secretion of gastric juice.
(2) The structure of the cells that secrete the juice.
(3) The composition of the juice itself.
(4) Its action upon food.

1. THE PHYSIOLOGY OF THE SECRETION OF GASTRIC JUICE

Dr. Beaumont writes as follows :—'The inner coat of the stomach in its natural and healthy state is of a light or pale pink colour, varying in its hues according to its full or empty state. It is of a soft or velvet-like appearance, and is constantly covered with a thin, transparent, viscid mucus lining the whole interior of the organ. Immediately beneath the mucous coat, and apparently incorporated with the villous[5] membrane, appear small spheroidal or oval-shaped granular bodies from which the mucous fluid appears to be secreted. On the application of aliment the size of the vessels is increased, the colour heightened, and vermicular movements excited. The gastric glands begin to discharge a clear, transparent fluid, which continues rapidly to accumulate as aliment is received for digestion. This fluid is invariably distinctly acid. The mucus of the stomach is less fluid and more viscid, and sometimes a little saltish, but does not possess the slightest character of acidity. On applying the tongue to the mucous coat of the stomach in its empty, unirritated state, no acid taste can be perceived. When food or other irritant has been applied to the membrane, the acid taste is immediately perceptible.'

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[1] The most important facts made out by Dr. Beaumont are detailed in Dr. Lauder Brunton's book, *Disorders of Digestion*, 1886.

[2] *Compt. rend.* lxxxiv. 1514; lxxxv. 156.

[3] *Ann. Chem. Pharm.* xcii. 42.

[4] *Diss.* Dorpat, 1853.

[5] The use of the word *villous* here is erroneous; the stomach has no villi.

This simple statement really contains the essence of all our subsequent knowledge on the subject. When not excited the stomach is free from gastric juice; it may be excited, as it normally is, by the presence of food; but mechanical, thermal, chemical, or electrical stimuli may also be employed. Dilute alkalis, such as the saliva, excite the secretion especially well. Schiff[1] made the statement that active gastric juice is only secreted after absorption of what he termed peptogens: dextrin was one of the most important of these; soup was another. Schiff's method of experimentation is open to some question,[2] but these substances do undoubtedly act as excitants of the secretion. Schiff's mistake was to attribute to them the sole power of exciting gastric secretion. There is no doubt that purely nervous (reflex) mechanisms come also into play. Thus the smell, the sight, the thought of food will excite a flow of the juice. In Richet's case of gastric fistula (the œsophagus had been occluded by caustic alkali) the placing of sugar or lemon juice on the tongue caused a secretion of gastric juice; in this case no saliva could have reached the stomach. Cl. Bernard once observed in a dog a flow of the juice on excitation of the vagus nerve. He also once observed that stimulation of the sympathetic nerves passing from the semilunar ganglia to the stomach arrested the secretion. Rutherford has shown that when the vagi are cut during digestion, the lining of the stomach becomes pale; that stimulation of the peripheral end produces no effect, but that stimulation of the central end causes the mucous membrane once more to be reddened. In rabbits, however, after division of both vagi below the œsophagus, digestion goes on in a normal way.

What is to be learnt from a number of observations of this kind?

First, that our knowledge is most inexact, and that thorough and consecutive experiments are much to be desired. Secondly, that though inexact they at least teach the fact that the nervous system has some control over gastric secretion. Thirdly, that any direct influence of nerves on gastric secretion, as in the case of the salivary glands, has not been discovered. Fourthly, that what nervous influence has been discovered is exerted rather on the blood-vessels than on the secreting cells of the stomach, the increased flow of gastric juice being secondarily produced by the dilatation of the blood-vessels. We, in fact, possess a considerable amount of knowledge concerning the vaso-motor nerves of the abdominal organs, and also of the nervous mechanism of peristalsis; but in a work on chemical physiology we have only to do with these to a very small extent. A theory has been promulgated that local centres in the ganglia of the plexuses of the stomach and intestine have an influence on both blood-vessels and secretion. Such a theory would require very forcible backing up before it could be regarded as tenable. All recent research goes to prove the relatively small importance of peripheral centres for the carrying out of reflex actions.

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[1] *Archives des sciences physiques et naturelles*, 1877.

[2] Langley, *Journ. Physiol.* iii 291.

2. THE STRUCTURE OF AND CHANGES IN THE CELLS THAT SECRETE GASTRIC JUICE

Two kinds of glands are distinguished which differ from one another in the character of their enclosed cells, and in the nature of their secretion. The *pyloric glands* are so called because they are found most numerously in the pyloric region; they are distinguished by the large size and depth of the gland mouth or duct as compared with the tubules that open into it. The duct is lined by columnar cells continuous with and similar to the columnar epithelium covering the general internal surface of the stomach; the tubules are lined with shorter and more cubical cells, which are uniformly granular throughout. The *cardiac glands* (fundus glands of Heidenhain) are so called because they occur most numerously in the cardiac half of the stomach. Their duct is short, their tubules, in proportion, long. The latter are filled with polyhedral cells, only a small lumen being left; they are more coarsely granular than the corresponding cells of the pyloric glands. These cells were called *principal cells* by Heidenhain,[1] *adelomorphic cells* by Rollett,[2] and *central cells* on account of their position. Between them and the basement membrane of the tubule are other cells of a different nature called *parietal cells* (Heidenhain), *delomorphic cells* (Rollett), or *oxyntic cells* (Langley).[3] They are most numerous in the more superficial portions of the tubules. Their granular appearance is due to a close and uniform intercellular network (Klein).[4] They are readily stained by many colouring agents, especially aniline blue.

The changes that occur in these different cells on secretion have been worked at by Heidenhain, Ebstein,[5] and Langley.

The following is in brief the substance of Langley's observations:—

The use of osmic acid is to be much recommended for studying these conditions, as hardening reagents like alcohol cause the granules to become swollen and indistinct.

The central cells exhibit changes similar to those already described as occurring in the salivary glands. Before secretion they are 'loaded' with granules; during secretion they discharge their granules, those that remain being chiefly situated near the lumen, leaving in each cell a clear outer zone (*see* fig. 84).

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[1] *Arch. f. mikr. Anat.* vi. 368.

[2] *Centralbl. med. Wiss.* 1870, Nos. 21 and 22.

[3] *Journ. of Physiol.* ii. and iii. (ὀξὺς = acid). They were formerly called peptic cells, a term that must now be discarded.

[4] *Stricker's Handbuch*, 1871.

[5] *Arch. f. mikr. Anat.* vi.

The cells of the pyloric glands undergo similar changes. In both these cells and the central cells of the cardiac glands some substance readily precipitable by alcohol makes its appearance during discharge, as this reagent then renders the cells turbid.

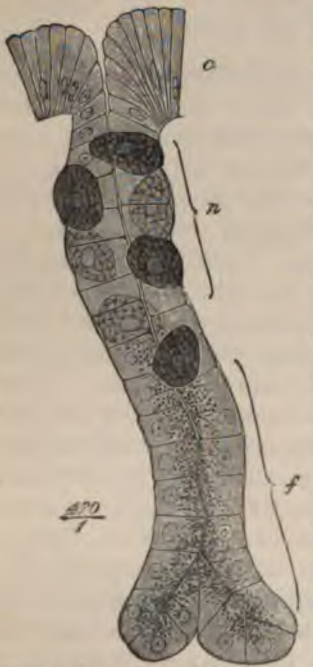

FIG. 84.—A Cardiac Gland of simple form from the Bat's Stomach. Osmic acid preparation (Langley). *c*, columnar epithelium of the surface; *n*, neck of the gland, with central and parietal cells; *f*, base or fundus, occupied only by principal or central cells, which exhibit the granules accumulated towards the lumen of the gland.

The oxyntic cells undergo merely a change of size during digestion, being at first somewhat enlarged and then shrinking to less than their original volume (Heidenhain).

We have in the granules of the central cells another instance of a zymogen or ferment-precursor. It is the precursor of pepsin, and is called *pepsinogen*. The parietal cells are those which secrete the hydrochloric acid. The evidence upon which this statement rests is the following: Heidenhain by means of a surgical operation, performed antiseptically, succeeded in making in one dog a *cul-de-sac* of the fundus, in another of the pyloric region of the stomach; the former secreted a juice containing both acid and pepsin; the latter, parietal cells being absent, secreted a viscid alkaline juice containing pepsin.

Brücke showed that the acidity of the glands is greatest near their mouth; here also the parietal cells are most abundant; and no doubt the acid is quickly expelled from the glands. Cl. Bernard showed this by his well-known experiment of injecting potassium ferrocyanide in one vein of an animal, and lactate of iron into another. These substances in presence of free acid strike a blue colour, and he found only the surface of the mucous membrane of the stomach was blue. In the frog there is a well-marked separation of two regions: the œsophageal region, free from parietal cells, secretes an alkaline juice; the stomach itself, which contains the parietal cells, an acid juice (Langley).

Thus, although there can be but little doubt that the central cells secrete pepsin, the argument that the parietal cells secrete acid is at present one of exclusion only.

The rennet-ferment (rennin or chymosin) appears to be formed by the same cells that manufacture pepsin. Hammarsten[1] and Langley[2] obtained evidence of the existence of a zymogen of rennin analogous to that of pepsin or ptyalin; a weak alkaline extract of the mucous membrane contains no rennet; a weak acid extract contains rennet, and causes clotting in milk, even if the extract be made alkaline. A weak acid is generally found effective in converting a zymogen into a zyme or ferment.

The secreting cells of the stomach, like secreting cells universally, select certain materials from the lymph which bathes them; these materials are worked up by the protoplasmic activity of the cell into the secretion which is then discharged by the cell into the lumen of the gland of which it forms part. The most important substance in a digestive secretion is the ferment; in the case of the gastric juice this is pepsin; we can trace an intermediate step in the process by the visible presence of its precursor, pepsinogen. But another equally important material in the juice is the acid, for pepsin acts only in acid media. We have now, therefore, to consider a little more fully the differences between pepsin and pepsinogen, and, secondly, the important but puzzling problem of the formation of a free acid from the alkaline blood or lymph.

Pepsin and pepsinogen.—The following research was carried out by Langley and Edkins.[3] Their object was to discover a method of determining the relative amounts of pepsin and pepsinogen in any given fluid, and thence to determine whether both exist in the gastric glands. The following two methods were found to give approximate results:—

(1) The power of sodium carbonate to destroy pepsin is much greater than its power to destroy pepsinogen. Thus if equal volumes of neutralised acid extract of gastric mucous membrane and 1 per cent. sodium carbonate solution be mixed, $\frac{9}{10}$ to $\frac{19}{20}$ of the pepsin is destroyed in fifteen seconds, and it is unable to digest such a proteid as fibrin.

(2) The power of carbonic acid to destroy pepsinogen is greater than its power to destroy pepsin. If an aqueous extract of a frog's œsophagus be taken, and a stream of the gas passed through it for half an hour, $\frac{19}{20}$ to $\frac{59}{60}$ of the digestive power of the fluid is destroyed; while if an aqueous extract be warmed with dilute acid in the first instance, to convert the pepsinogen into pepsin, and it is then neutralised and the gas passed through it, there is little or no loss of digestive power. The passage of carbonic acid through the extracts throws down a precipitate of a globulin; but pepsinogen, which is thus probably a globulin, is not carried down unaltered, since a solution of the precipitate in dilute hydrochloric acid has little or no digestive power. Pepsinogen and pepsin are both destroyed at 54° to 57°, the temperature at which the globulin is coagulated.

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[1] *Maly's Jahresb.* 1872, p. 123.

[2] *Journ. of Physiol.* iii. 287.

[3] *Ibid.* vii. 371.

The destruction of pepsinogen by carbonic acid is increased by the presence of a small amount of neutral salt, and diminished by small amounts of peptone.

The gases oxygen and carbonic oxide have no effect on either pepsinogen or pepsin.

On applying the above methods to the œsophageal glands of the frog, it was found that little or no pepsin is present in the cells themselves. The conversion of pepsinogen into pepsin that occurs when the secretion leaves the cells is, no doubt, the same chemical change as that produced by the action of a dilute acid on the zymogen.

The formation of hydrochloric acid.—There is at present no thoroughly satisfactory theory to account for the presence of free hydrochloric acid in the gastric juice. Foster[1] suggests that it may be formed by the decomposition of some highly complex and unstable chlorine compound formed in the cell by union of organic substances with the chlorine of sodium chloride. Most other observers have considered that sodium chloride is a more direct source of the acid; but sodium chloride is, as Foster points out, an exceedingly stable substance, and carbonic acid, the only free acid in the blood, is a weak acid. The terms weak and strong as applied to acids are, however, misleading. So-called weak acids are, by what is termed 'mass influence,' able to unite with bases, displacing acids of greater 'avidity.' Thus the formation of free hydrochloric acid from sodium chloride and carbonic acid is not only a possible, but probably the correct explanation of the phenomenon (Bunge, 'Physiol. Chem.' p. 161). Ralfe attributes the production of the acid to the passage of electric currents through the mucous membrane, causing a reaction between sodium bicarbonate and sodium chloride, thus: $NaHCO_3 + NaCl = Na_2CO_3 + HCl$, but there are no valid grounds for supposing that such currents exist. It appears to me more probable that it is lactic acid which is chiefly instrumental in the decomposition of sodium chloride. Lactic acid is generally found in the stomach during a meal, especially if the meal contains carbohydrates; fermentative changes in these produce the lactic acid, which reacting with the sodium chloride produces sodium lactate and hydrochloric acid. This view was first promulgated by Maly.[2] Lactic acid certainly will decompose sodium chloride in this way in cold dilute solutions. Drechsel has discovered that the lactates in the blood are increased from 0·01 to 0·02 per cent. during digestion; a fact that supports Maly's view of the case. The great difficulty, however, in accepting Maly's theory is that carbohydrates are not always present in the food, and that a flow of acid from the gastric glands can be excited by distilled water or mechanical irritation. What, then, is the source of the lactic acid under those circumstances? This objection is met by Landwehr[3] by the following ingenious theory, in which animal gum (p. 480) plays an important part: the lumen of the gastric glands is always more or less filled with mucus; when the glands are stimulated a ferment is produced which decomposes the mucin, forming lactic acid from its carbohydrate constituent (animal gum); this acid reacting on sodium chloride produces free hydrochloric acid and sodium lactate: the former is poured into the stomach; the latter is absorbed by the blood. If it be admitted that sodium chloride is a direct source of hydrochloric acid, Landwehr's theory of the *modus operandi* appears to be a satisfactory one. It is, however, possible that the sugar of the blood and lymph is the real source of the acid.

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[1] *Text-book*, 5th edit. p. 419.

[2] *Sitzungs. d. Wien. Akad.* vol. lxix. 1874; also vol. lxxvi. In the latter paper a further suggestion is made, viz. the acid originates by the interaction of the sodium chloride and the sodium dihydrogen phosphate of the blood.

[3] *Chem. Centralbl.* 1886, p. 484; *Pflüger's Archiv*, xl. 21.

An attempt to solve the question was made by Külz[1]; he administered bromides and iodides, and then sought for free hydrobromic or hydriodic acid respectively in the gastric juice, and found it. As Drechsel[2] points out, however, the decomposition might have been effected by the hydrochloric acid of the juice, and not by the metabolic activity of secreting cells. If chlorides are not given in the food, hydrochloric acid disappears from the gastric juice after a time (Cahn[3]).

It is found that as the acidity of the gastric juice increases, that of the urine diminishes. This is not because of any diminution of free acid in urine—as urine contains no free acid—but because the amount of the base liberated by the formation of the gastric acid is increased, and passes into the urine. If sodium lactate is produced it no doubt is changed into sodium carbonate, which passing into the urine tends to render it alkaline.

3. COMPOSITION OF GASTRIC JUICE

The methods of obtaining gastric juice that have been adopted are the following :—

Spallanzani[4] fed birds on pieces of sponge to which a piece of string was attached ; after the sponge had remained in the stomach for a sufficient length of time to absorb the juice, it was pulled up by means of the string.

Since then gastric juice has been obtained from cases of gastric fistulæ both in men and animals. The first case carefully observed in a human being was that of Alexis St. Martin ; the first artificial gastric fistula in dogs was made by Blondlot ;[5] Bardeleben,[6] Bidder and Schmidt,[7] Bernard,[8] Holmgren,[9] Panum,[10] and many others have since then performed similar experiments.

For the investigation of the action of the gastric juice, it has been found that artificial gastric juice acts in the same way as the genuine article, and it is much easier to obtain. Schwann was the first to make an artificial juice, by extracting the mucous membrane of the stomach of a recently killed dog with 0·2 per cent. hydrochloric acid ; v. Wittich was the first to make a glycerin extract of the mucous membrane. The mucous membrane must be allowed to stand twenty-four hours before the extract is made, or treated with a little dilute hydrochloric or acetic acid, or with solution of sodium chloride.[11] By either of these means pepsinogen is converted into pepsin; glycerin is then added, and allowed to extract the pepsin for at least eight days. For artificial-digestion experiments, an artificial juice may be made by mixing a little of this extract with 0·2 per cent. hydrochloric acid; or, better, the pepsin is precipitated from the extract by means of alcohol; this precipitate is collected and dried at a low temperature, and when required dissolved in hydrochloric acid of the same strength.

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[1] *Zeit. Biol.* xxiii. 460.

[2] *Ibid.* xxv. 396.

[3] *Zeit. physiol. Chem.* x. 522.

[4] *Versuch. über das Verdauungsgeschäft, übers. von Michaelis*, Leipzig, 1785.

[5] Blondlot, *Traité analytique de la digestion*, Paris, 1843.

[6] *Arch. f. physiol. Heilk.* 1849, vol. viii.

[7] *Die Verdauungsäfte und der Stoffwechsel*, Mitau and Leipzig, 1852.

[8] Bernard, *Leçons de physiol. expérimentale*, Paris, 1856.

[9] Virchow-Hirsch, *Jahresb.* 1869, p. 103.

[10] *Ibid.* 1879, p. 99.

[11] Grützner, *Neue Unters. ü. d. Bildung des Pepsin*, Breslau, 1875.

The gastric juice in those animals in which it has hitherto been examined is either colourless or faintly yellow, clear, not viscid, and acid. It does not coagulate on boiling, but gives abundant precipitates with lead acetate, with mercuric chloride and with alcohol, showing the presence of pepsin; it gives no precipitate with acetic acid, showing the absence of mucin.

The two important constituents of the juice, the acid and the ferment, have already been several times mentioned, and we have endeavoured to trace the manner of their formation by the cells that secrete them. We have now to take up the question of their quantity in the juice itself, the methods of detecting them, and separating them from the other constituents of the juice. Before passing, however, to the consideration of these subjects, the following table of analyses may be first given :—[1]

Constituents	In parts per 1000		
	Human	Dog	Sheep
Water	994·404	973·062	986·143
Organic substances (chiefly pepsin)	3·195	17·127	4·055
HCl	0·200	3·050	1·234
$CaCl_2$	0·061	0·624	0·114
NaCl	1·465	2·507	4·369
KCl	0·550	1·125	1·518
NH_4Cl	—	0·468	0·473
$Ca_3(PO_4)_2$	0·125	1·729	1·182
$Mg_3(PO_4)_2$		0·226	0·577
$FePO_4$		0·082	0·331

The points to be noted in such a table are the following :—

1. The relatively low percentage of solids in the human juice as compared with that of the other animals, particularly the dog. The young woman from whom the juice was removed is spoken of as healthy; this term is a comparative one; there appears to be little doubt that

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[1] The above table was constructed by C. Schmidt and his pupils (Bidder and Schmidt, *Ann. Chem. Pharm.* xcii. 42). The case of human fistula from which the juice was obtained was that of a healthy young woman named Katherina Kutt under Grünwaldt's care.

in a perfectly healthy person, that is, a person without a gastric fistula, the percentage of both pepsin and acid would be higher, though of course not so high as in a carnivorous animal like the dog.

2. The great preponderance of chlorides over other salts; apportioning the total chlorine found to the various metals present, that which remains over must be combined with hydrogen to form the free acid of the juice.

The acids of the gastric juice.—Beaumont by the tongue, and C. Schmidt by litmus paper, demonstrated the fact that the stomach when not secreting was alkaline, but that the juice it poured out on stimulation was acid. In order to guard against error from the occurrence of acids introduced with or formed from food, Brücke neutralised the contents of the stomach with magnesia, and on removing this squeezed the stomach and obtained an acid juice. Cl. Bernard's experiment with potassium ferrocyanide and lactate of iron has been already mentioned (p. 634).

Prout[1] was the first to obtain hydrochloric acid by the distillation of the gastric juice. Dunglinson and Emmett obtained the same result from the juice in Dr. Beaumont's case. Lehmann[2] considered that this result was due to the action of lactic acid on the chlorides in the juice, and not to the presence of free hydrochloric acid. Leuret and Lassaigne[3] supported this view, as they found free lactic acid in the stomach. The analyses by C. Schmidt, however, placed the matter on a safe footing, as he showed that the amount of chlorine was greater than would combine with the metals and ammonium present in the juice.

There is, however, very little doubt that, though hydrochloric acid is the acid *par excellence* of the juice, lactic acid does occur during digestion; this consists partly of sarcolactic acid derived from meat, and fermentation lactic acid derived from carbohydrates. The amount of lactic acid is much increased in those disordered conditions of the stomach when excessive fermentative processes are occurring. Small quantities of volatile acids, such as acetic and butyric, are also produced in this way.

Numerous methods have been devised for the detection of these acids, and the most important of these are the following:—

1. For free hydrochloric acid. The tropæolin test has been already described (p. 629); other colour reactions extensively used are as follows: Solutions of gentian-violet or methyl-violet are turned blue: this is exceedingly delicate, but is hindered by the presence of peptone. Uffelmann,[4] who has devoted a large

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[1] *Phil. Trans.* 1824, p. 45.

[2] *Ber. d. Sächs. Gesell. d. Wiss.* Leipzig, i. 100.

[3] *Recherches physiol. et chim. de la digestion*, Paris, 1825.

[4] *Zeit. klin. Med.* viii. 392.

amount of attention to this subject, recommends one of the two following reactions: (*a*) 0·5 c.c. of red Bordeaux wine is mixed with 3 c.c. of alcohol and 3 c.c. of ether; the mixture is almost colourless, and gives a rose colour with a few drops of a 0·45 to 0·5 per 1000 solution of hydrochloric acid, even in the presence of peptone, albumin, and salts. (*b*) Bilberries are made into a pulp with water, extracted with amylic alcohol, and the extract used for colouring paper, which is thereby turned blue or greyish-blue, fainter than blue litmus. When this is dipped into 0·2 per 1000 hydrochloric acid it is turned red. Lactic, acetic, and butyric acids give the same reaction when present in the proportion of 4 to 6 per 1000; a proportion never found in the contents of the stomach. Wiesner and Singer have introduced a reagent consisting of 2 parts of phloroglucinol, 1 part of vanillin, and 30 parts of rectified spirit. A few drops of filtered gastric juice is evaporated with an equal quantity of the reagent and red crystals, or if much peptone is present, a red paste is formed. The reaction takes place with 1 part hydrochloric acid in 10,000.[1] The organic acids do not give this reaction.

2. For free lactic acid. A solution is made by mixing 10 c.c. of 4 per cent. carbolic acid with 20 c.c. of water and 1 drop of the liquor ferri perchloride of the British Pharmacopœia. An amethyst blue clear liquid is formed, which is turned yellow by lactic acid when present in only 1 part per 10,000. The test is best performed as follows: Boil the stomach contents and filter; extract the filtrate with ether; evaporate the ethereal extract to dryness, dissolve the residue in a little water, and add a few drops of the reagent (Uffelmann). Hydrochloric acid simply renders the fluid colourless, and must be present in fairly large quantities to do this.

The methods devised for estimating the amount of the acids in the stomach are the following:—

The oldest method is that of Bidder and Schmidt: this consists in performing an ultimate analysis, apportioning the chlorine to the metals and ammonium present, and calculating the remainder as hydrochloric acid.

Rabuteau's method [2] modified by Cahn and v. Mering,[3] consists in driving off the volatile acids by heat and shaking the residue with a large excess of ether, which takes up the lactic acid: this is separated, and cinchonine (Rabuteau used quinine) is added to the remainder until the reaction is neutral. The cinchonine hydrochloride is dissolved out by shaking with chloroform; the chloroform is distilled off from this extract, and chlorine estimated in the residue.

Another method, devised by Cahn and v. Mering, consists in distilling the contents of the stomach with water three times. The volatile acids are estimated in the distillate. The residue is shaken six times with 500 c.c. of ether, and this is evaporated to dryness, and the lactic acid estimated by titration. The residue contains the hydrochloric acid, and this also can be estimated by titration (see Acidimetry, p. 16).

Another method, used by Seemann[4] and Hehner,[5] consists in neutralising the stomach contents by titrating with sodium hydrate, evaporating to dryness, and carefully incinerating. The ash is extracted with water, and the alkali present in the extract is estimated by titrating with an acid; the difference between the amount of alkali added and the amount of alkali found gives the amount which must have combined with hydrochloric acid, the lactic and volatile acids being burnt during incineration. This method gives results a little too high; the other methods take a long time and a large quantity of reagents.

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[1] A. Günsberg, *Chem. Centralbl.* 1887, p. 1560.

[2] *Comptes. rend.* lxxx. 61.

[3] *Deutsch. Arch. klin. Med.* xxxix. 329.

[4] *Zeit. klin. Med.* v.

[5] *Zeit. anal. Chem.* xvii. 236.

A method introduced by Sjöqvist[1] gives, according to him, perfectly accurate results, and is sufficiently simple to use clinically, as in the examination of vomit. The contents of the stomach are evaporated to dryness with barium carbonate, and then incinerated; barium chloride is thus formed and remains unchanged, but the barium salts of the organic acids are burnt to barium carbonate. The barium chloride is then extracted with water, and the quantity of this salt which goes into solution is a measure of the original amount of free hydrochloric acid. The titration is carried out as follows: The solution of barium chloride is placed in a beaker, and a quarter of its volume of alcohol added, then a few c.c. of a 10 per cent. solution of sodium carbonate containing 10 per cent. of acetic acid. A standard solution of potassium dichromate (of which 1 c.c. corresponds to 4·05 milligrammes HCl) is then added from a burette till the end reaction is obtained; the burette is read, and the quantity of HCl calculated. The indication of the end of the reaction is the yellow colour which the smallest excess of the dichromate gives to the liquid floating over the white precipitate produced by the interaction of the two salts. A more delicate test for excess of dichromate is, however, Wurster's tetramethylparaphenylene-diamine paper. Potassium dichromate in an acetic acid solution acts in the same way as ozone, to test for which the paper was originally used; it turns it blue.

The ferments of the gastric juice. Pepsin.—The name pepsin was given to the proteolytic ferment of the gastric juice by Schwann.[2] Wasmann[3] was the first who attempted to isolate it, E. Brücke[4] the first who succeeded. Brücke's method consists in extracting the mucous membrane of the stomach with a 5 per cent. solution of phosphoric acid; to this lime-water is added, and the precipitate of calcium phosphate so formed carries the pepsin down with it. The precipitate is collected on a filter, washed with water, and dissolved in dilute hydrochloric acid; to this solution a saturated solution of cholesterin in a mixture of alcohol and ether (4 : 1) is added in small quantities; the cholesterin is precipitated, and this, again, carries down the pepsin with it. The precipitate is washed first with a weak solution of acetic acid, then with water, and lastly with ether. The ether dissolves out the cholesterin and leaves the pepsin undissolved. The pepsin, which by this method is obtained only in small quantities, is then dried at a low temperature.

Von Wittich's[5] method of precipitating the pepsin by alcohol from a glycerin extract of stomach gives a larger yield. This is freed from salts and peptones by dialysis.

The constitution of pepsin is unknown. The elementary analyses of it that have been made [C, 53; H, 6·7; N, 17·8; O, 22·5 (Schmidt);

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[1] *Zeit. physiol. Chem.* xiii. 1.

[2] *Arch. f. Anat. u. Physiol.* 1836, p. 90; *Pogg. Ann.* xxxviii. 358.

[3] *Diss.* Berlin, 1839.

[4] *Sitzungsber. Wien. Akad.* xliii. 602.

[5] *Pflüger's Archiv*, iii. 193.

C, 51 ; H, 7·2 ; N, 15·4 (Chapoteaux)] yield numbers approximately the same as proteids ; the observations of Langley and Edkins show also that the temperature at which solutions of pepsin lose their activity (57° to 58°) is the same as that at which the proteid in solution is coagulated. Probably pepsin, like other enzymes, is either a proteid or a proteid-like substance. Pepsin can be heated to 100° in the dry condition without losing its power.[1] By standing under dilute alcohol or by precipitation with metallic salts, it does not lose its fermentative activity. Strong alcohol in time destroys its power. It does not dialyse through animal membranes nor through parchment paper. Brücke states that it is precipitated from an aqueous solution by lead acetate and platinum chloride, but not by silver nitrate, tannic acid, acetic acid, and potassium ferrocyanide, nor does it give the xanthoproteic reaction.

Pepsin is active only in an acid medium. It has been surmised that pepsin and the acid form a loose compound.[2] Other acids can take the place of hydrochloric acid, but are less favourable to the action of the ferment ; nitric acid (0·1 to 0·4 per cent.) and lactic acid are next best; sulphuric, phosphoric, acetic, formic, &c. follow at a long interval.[3] Pepsin is most energetic at a temperature a little above that of the body (40° C.). Fick and Murissier,[4] and also Hoppe-Seyler,[5] are inclined to believe that the pepsin of cold-blooded animals is somewhat different from that of the warm-blooded animals, as it does not work more energetically at 40° C. than at 0° C.

The fate of pepsin.—Like other ferments, pepsin is not exhausted by the work it does, but is always available to perform more. Some of it is doubtless absorbed, as it is found in the tissues (*see* p. 412) and in the urine. The pepsin which passes on into the small intestine is rendered inactive by the alkalinity of the juices there, and according to Langley [6] is destroyed by the trypsin of the pancreatic juice.

The rennet ferment.—Hammarsten [7] states that pepsin and rennin are different ferments for the following reasons :—

(*a*) Rennin is destroyed by a lower temperature than pepsin.

(*b*) Though both are precipitable either by magnesium carbonate or lead acetate, the precipitation of pepsin is complete, that of rennin incomplete. By fractional precipitation they can thus be separated.

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[1] Al. Schmidt, *Centralbl. med. Wiss.* 1876, No. 29.

[2] Chandelon, *Bull. de l'acad. royale*, 1887, vol. i. p. 289.

[3] Davidson and Diebrich, *Arch. f. Anat. u. Physiol.* 1860, p. 688; Wolffhügel, *Pflüger's Arch.* vii. 188 ; Ebstein and Grützner, *Ibid.* viii. 132.

[4] *Verhandl. d. Würzburg. phys. med. Ges.* N.F. iv. p. 120.

[5] *Pflüger's Archiv.* xiv. 394.

[6] *Journ. Physiol.* iii. [illegible]

[7] Virchow-Hirsch, *Jahresb.* 1873, p. 133.

4. THE ACTION OF GASTRIC JUICE

The action of gastric juice can be readily demonstrated by a simple experiment. Four test-tubes are taken which we may label A, B, C, and D. A is half filled with water and a few drops of a glycerin extract of stomach added to it, or a few fragments of pepsin dissolved in it; B is half filled with 0·2 per cent. hydrochloric acid; C is half filled with a solution of pepsin in 0·2 per cent. hydrochloric acid, or a few drops of a glycerin extract of stomach may be added to half a test-tube full of 0·2 per cent. hydrochloric acid; D is half filled with the same liquid. A small fragment of a solid proteid, such as a piece of lean meat or a shred of fibrin, is placed in A, B, and C; D is filled up with a 10 per cent. solution of egg-albumin.

All four test-tubes are now put in a warm bath at 40° C., and after about ten to twenty minutes they may be taken out and examined. In A, which contains pepsin alone, the fibrin is unaltered; in B, which contains hydrochloric acid alone, the fibrin is swollen and transparent (with rather stronger acids, acid-albumin would be formed, or even small quantities of albumoses); in C, containing both acid and pepsin, the fibrin will be swollen and partly dissolved; a little later it will be almost entirely dissolved, the products being acid-albumin, albumoses, and peptone. In D there will be no visible change, but on testing for albumin little or none would be found, it also being transformed into acid-albumin, albumoses, and peptone. On prolonged digestion the acid-albumin is converted into peptone; a somewhat insoluble product called anti-albumid is, however, first formed; the albumoses are finally converted into peptones too.

The following simple tests will show the presence of the products of gastric digestion:—

(*a*) Colour a small quantity of the liquid with litmus, and neutralise with dilute (0·1 per cent.) alkali; a precipitate of acid-albumin or syntonin is produced, which dissolves in excess of alkali.

(*b*) Add nitric acid; a precipitate of the albumoses is produced, which dissolves up on heating, and reappears on cooling.

(*c*) Add a drop of very dilute copper sulphate solution; a precipitate is produced; this dissolves up on adding ammonia, forming a violet solution, or on adding potash or soda forming a rose-red solution. This so-called *biuret reaction* is given by both peptones and albumoses. Ordinary proteids (albumins and globulins) give a blue solution with copper sulphate and ammonia, and a violet with copper sulphate and potash or soda. This test should always be performed in the cold.

(*d*) Saturate the liquid with ammonium sulphate (after neutralisation) ; a precipitate is produced ; filter ; the precipitate consists of the albumoses ; the filtrate contains the peptone, which gives no precipitate on boiling ; no precipitate with nitric acid ; it, however, gives a yellow colour on being heated with nitric acid, which is turned orange by ammonia ; it also gives the biuret reaction. (When ammonium sulphate is present, a large excess of potash is necessary to get the red tint.) The distinctive and useful feature of a peptone is that, unlike other proteids, it is readily diffusible through an animal membrane, and thus proteids can be absorbed. The peptones are undoubtedly the products of the hydration of proteids ; they and their intermediates, the albumoses, can be formed by other hydrating agents, such as dilute mineral acids or superheated steam. It has also been recently stated that dehydrating agents will produce ordinary proteids from peptone.[1]

The earliest experiments of this nature were made by Schwann[2] and Lehmann.[3] Before their time digestion experiments had been performed in various other ways ; the ancients supposed that the breaking up of the food was effected by the stomach grinding down the food in the same manner as the gizzard of a bird ; others had an idea that heat and moisture in the stomach produced a kind of putrefaction. Réaumur in 1752, and Spallanzani in 1783, fed birds on materials enclosed in perforated metallic balls ; after a time these balls were vomited up, or in Spallanzani's experiments withdrawn by a string ; the examination of the semi-digested food showed them that mechanical grinding could not have produced the effect, and also that there was no odour of putrefaction. Then came the discovery that the juice secreted by the stomach was acid ; the first observers were inclined to attribute the solvent power of the juice to its acid, but, as Dr. Beaumont showed, an acid of the same strength is a less powerful solvent, and therefore the gastric juice must contain a special solvent principle ; this Eberle supposed to be the gastric mucus, a supposition easily refuted. It was Schwann who discovered this special principle and called it pepsin ; he gave the name *albuminose* to the product of its action on albumin ; Lehmann's name peptone, however, has since been generally adopted. Lehmann recognised that peptone is not coagulated by heat as albumin is. Meissner[4] described a number of products which he termed parapeptone, dyspeptone, metapeptone, *a*, *b*, and *c* peptone. Schmidt-Mulheim distinguished between parapeptone, propeptone, and peptone. Parapeptone is the acid-albumin ; propeptone is a very good name for what we now call the proteoses. Brücke[5] gave the names *hydrophyr* to a variety of peptone insoluble in alcohol, and

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[1] Bunge's *Physiol. Chem.* p. 201. [2] *Loc. cit.* [3] *Lehrbuch*, 1850.

[4] *Zeit. rat. Med.* vii., viii., x., xii., and xiv.

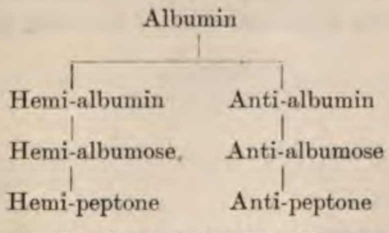
[5] *Sitzungsber. Wien. Akad.* xxxvii. 172 ; lxi. Abth. 2.

alkophyr to one stated to be soluble in alcohol; this apparent solubility arose from the fact that the alcohol used was not absolute. Moldenfeld,[1] Kossel,[2] Maly,[3] and Herth[4] made elementary analyses of the peptones they obtained.

Grützner[5] introduced a very valuable method of estimating the relative digestive activity of artificial gastric juices. A 0·1 per cent. solution of carmine is made with glycerin containing a little ammonia; this is used for staining finely divided fibrin, which is then well washed with water and preserved for use in ether. Equal quantities of the coloured fibrin are placed in equal quantities of the two liquids to be tested; as the fibrin is digested, carmine enters into solution, and the liquids are compared with one another, and with a standard solution of carmine after a given time, with regard to the intensity of their tint.

Nearly all of our present knowledge of the chemistry of digestion is, however, due to the work of Kühne[6] and those associated with him in his researches, particularly Chittenden[7] and Neumeister.[8] A most valuable method of isolating peptone was discovered by Wenz,[9] one of Kühne's pupils. It consists in the use of ammonium sulphate as a reagent; when added to saturation this neutral salt readily precipitates all proteids except peptones. Pure peptone was never obtained previous to this, but always more or less mixed with albumoses.

The earliest of Kühne's observations showed him that there are two varieties of peptone; *hemipeptone*, which is by the pancreatic juice further split into leucine, tyrosine, &c.; *antipeptone*, which resists this action: the precursors of these substances in the albumin molecule we may call hemialbumin and antialbumin; and the intermediate albumoses (*a*-peptone of Meissner; propeptone of Schmidt-Mulheim), *hemialbumose* and *antialbumose*; we thus speak of hemi- and anti-groups of digestion products. Leaving for the present the syntonin or parapeptone out of account, we may represent the changes that occur as follows:—



More recent observations have shown that albumoses may be classified in another way, according to the action of various reagents on them, into—

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[1] *Med. chem. Unters.* Heft 4.

[2] *Pflüger's Archiv*, ix. 438.

[3] *Ibid.* ix. 585.

[4] *Zeit. physiol. Chem.* i. 277.

[5] *Loc. cit.*

[6] *Verhandlungen des Naturhist. med. Vereins zu Heidelberg*, N.F. i. *et seq.*

[7] Kühne and Chittenden, *Zeit. Biol.* xx. and xxii.

[8] *Ibid.* xxiii. and xxiv.

[9] *Ibid* xxii.

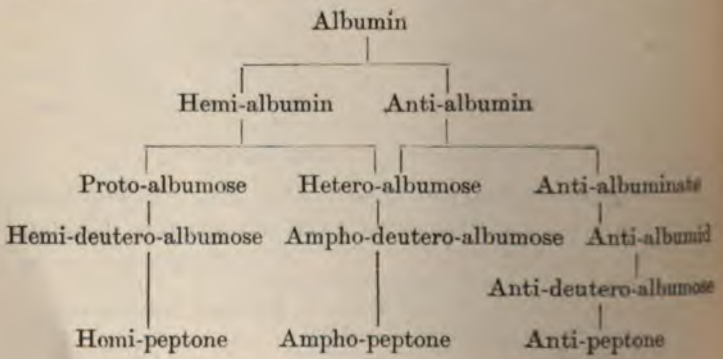
1. **Proto-albumose**: Soluble in hot and cold water, and in dilute saline solutions; precipitated by saturation with sodium chloride or magnesium sulphate.

2. **Hetero-albumose**: Insoluble in water, soluble in 0·5 to 15 per cent. sodium chloride solutions in the cold, but precipitated by heating to 65° C. The precipitate, however, is not a heat-coagulum, as it readily dissolves in dilute acid or alkali. Hetero-albumose is precipitated by dialysing out the salt from its solutions. Like the other albumoses, it is precipitated by alcohol, but unlike them it is partly converted into an insoluble product called dys-albumose. Hetero-albumose, like proto-albumose, is precipitated by saturation with neutral salts.

3. **Deutero-albumose**: Soluble in hot and cold water. It is not precipitated by saturation with sodium chloride or magnesium sulphate, but it is by saturation with ammonium sulphate. It is not precipitated by copper sulphate, and only gives the nitric acid reaction so characteristic of albumoses in the presence of excess of salt. It is thus in its reactions the nearest to the peptones.

The table on the next page contrasts the chief reactions of albumins and globulins (the native proteids) with those of the products of proteolysis (albumoses and peptones).

Further particulars concerning the separation and properties of these substances have been already given in the chapter on proteids (Chap. X). A table for their systematic separation is given on p. 14. For the results of elementary analysis, the original memoirs must be consulted. The question, however, which now especially concerns us is, how can the two classifications of digestion products be fitted in one with the other? Has each form of albumose an anti- and a hemi-variety, or are certain albumoses hemi- and certain others anti-? This question has been investigated by Neumeister, and the results of his investigations may be embodied in the following table:—



ety of oteid	Hot and cold water	Hot and cold saline solution, e.g. 10 per cent. NaCl	Saturation with NaCl or $MgSO_4$	Saturation with Am_2SO_4	Nitric acid	Copper sulphate	Copper sulphate and ammonia	Copper sulphate and caustic soda or potash
s . . .	Soluble in cold; coagulated in hot water	Soluble in cold, coagulated in hot solutions	Not precipitated	Precipitated	Precipitated in cold; not soluble on heating, or only slightly	Precipitated	Blue solution	Violet solution
s . . .	Not soluble in either	The same as albumins	Precipitated	Precipitated	The same as albumins			
bumose .	Soluble in both	Soluble in both	Precipitated	Precipitated	Precipitated in cold; precipitate dissolves with heat and reappears on cooling	Precipitated	Violet solution	Rose-red solution
albumose .	Insoluble, i.e. like globulins precipitable by dialysis from saline solutions	Soluble in both; partly precipitated, but not coagulated on heating to 65° C.	Precipitated	Precipitated	Ditto	Ditto	Ditto	Ditto
-albumose	Soluble	Soluble	Not precipitated	Precipitated	This reaction only ocurs in presence of excess of salt	Not precipitated	Ditto	Ditto
. . . . .	Soluble	Soluble	Not precipitated	Not precipitated	Not precipitated	Not precipitated	Ditto	Ditto

e scheme tabulated on the opposite page appears complicated, but iculties are more on the surface than real. The albumin may, ore, be considered to be composed of hemi-albumin and antiin ; the hemi-albumin in the first stage of hydration is split into albumose and hetero-albumose ; the anti-albumin yields heteroose and acid-albumin ; the acid albumin is called anti-albuminate table opposite, as it yields on subsequent digestion anti-products We thus see that deutero-albumose is not formed directly from the albumin, but is a second stage in the process of hydration. The albumoses formed directly from albumin (i.e. proto- and hetero-albumose) are called *primary albumoses*. Deutero-albumose is thus nearest to the peptones, not only in its reactions, but also in its method of formation.

If the three primary cleavage products of digestion be separated and subjected to further digestion, peptone is the ultimate result in each case. Proto-albumose yields hemi-peptone[1]; the deutero-albumose, which is an intermediate stage in the process, is termed hemi-deutero-albumose, as it yields hemi-peptone. Hetero-albumose indicating its double origin yields both anti- and hemi-, that is, ampho-peptone, the intermediate deutero-albumose being correspondingly named ampho-deutero-albumose. The anti-albuminate is first changed into the insoluble anti-albumid, which slowly yields anti-peptone, with an intermediate stage of anti-deutero-albumose.

Digestion in the stomach never goes further than the formation of peptone; leucine and tyrosine are not formed. Such is, so far as is at present known, the series of changes which albumin undergoes.

Fibrin is first dissolved, forming a solution of globulins (*see* p. 233), and these are then converted into the same series of products with peptone as the terminal product. The products of globulin may be termed globuloses; of vitellin, vitelloses; of casein, caseoses; of myosin, myosinoses; and these are very like the albumoses; any slight variations that may occur have been already alluded to in the descriptions already given of the separate proteids. They may be all conveniently grouped together under the general term proteoses. The products of digestion of elastin (p. 475) and of gelatin (p. 472) have a general resemblance to the proteoses and peptones. Mucin, keratin, and nuclein are not digested in the stomach. Hæmoglobin is split into hæmatin and acid-albumin; the former remains unchanged, the latter is digested. Except that the proteid envelopes of fat-cells are dissolved, fats are not altered in the stomach. Starch is also unaltered.[2] Cane sugar is said to be partially converted into dextrose and lævulose by the mucin of the stomach.[3]

We thus see that the stomach acts for all practical purposes on only one of the proximate principles of food, namely, the proteids.

Why the stomach does not digest itself during life is a question that has never yet received an answer. John Hunter's[1] view that it is due to a 'living principle' is no real explanation. The stomach after death may at a suitable temperature digest itself, and self-digestion may also occur in ulcerated portions of the stomach-wall where the circulation has been stopped, and so lead to perforation. But the mere alkalinity of the blood and lymph bathing the stomach is not the whole secret of its power of resisting the action of the acid juice; for the intestines and the pancreas are similarly able to withstand the digestive action of the pancreatic juice, which is most energetic in alkaline media.

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[1] Proto-albumose has never yet been obtained absolutely free from hetero-albumose; the purer it is, the less anti-product does it yield; Neumeister therefore concludes that if it were entirely free from impurity it would yield a pure hemi-peptone.

[2] Except in the stomachs of such animals as the horse and pig, where an alkaline juice is secreted for the first hour or so after the arrival of food (*see* pp. 612, 628).

[3] This action is, however, very slight (Komanos, *Diss.* Strasburg, 1875).

Pathological and other abnormal conditions of gastric digestion.—We have seen the conditions under which gastric digestion is most favourably carried on; we have now to see those which hinder or interfere with, or accompany, disorders of digestion in the stomach.

All salts of the heavy metals, such as mercuric chloride or lead acetate, which form precipitates with pepsin and proteids, completely stop both artificial and natural digestion. Concentrated solutions of alkaline salts (sodium or magnesium sulphate, &c.), which produce transudation of fluid into the stomach, act similarly; and quite small percentages of such salts (such as 0·004 per cent. of sulphate of sodium, potassium, ammonium, or magnesium, 0·02 of various urates, 0·01 of sodium phosphate, &c.) have a very considerable inhibitory effect when acid solutions of pure pepsin are used in experiments on artificial digestion; the same is true for trypsin (Nasse,[2] Heidenhain,[3] A. Schmidt,[4] E. Stadelmann[5]). Phenol must be somewhat concentrated to stop gastric digestion.[6] Bitters, according to Buchheim, do not further gastric digestion; but common experience is against this; like pepper they stimulate the mucous membrane to secrete.

An admixture with the gastric juice which is not uncommon in pathological states is bile. If this enters the stomach from the intestine, it precipitates the proteids, and prevents them entering that swollen condition so essential for gastric digestion. It also neutralises the gastric juice and thus entirely stops the activity of pepsin. Should, however, the pancreatic juice enter with the bile, it may happen that pancreatic digestion occurs in the stomach.[7]

To speak of all the varied pathological conditions of the stomach would be beyond the scope of this work. Our knowledge is in many cases the result of inference from the good or bad effects of certain methods of treatment; except in those cases where there is vomiting or a fistula, we can but rarely examine the actual stomach contents.

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[1] *Phil. Trans.* 1772.

[2] *Pflüger's Archiv*, xi.

[3] *Ibid.* x.

[4] *Ibid.* xiii.

[5] *Zeit. Biol.* xxv. 208.

[6] Buchheim, *Beiträge z. Arzneimittellehre*, Leipzig, 1849, Heft i. p. 112.

[7] Hoppe-Seyler, *Physiol. Chem.* p. 283.

The two chief forms of dyspepsia are (1) the *atonic*, where there is, as Beaumont observed, a condition of the stomach much resembling the furred tongue of the patient. The secretion is scanty; this is often owing to anæmia, and an imperfect blood-supply may lead to ulceration of the stomach-walls; fever also, as Beaumont showed, produces much the same effect; even mechanical stimulation of the stomach calls forth little or no secretion. (2) *Irritative dyspepsia*; in this the stomach is in a state of active congestion; the blood-supply is greater than normal; an excess of fluid is poured out into the stomach; but in these catarrhal conditions the fluid is not gastric juice, but an alkaline transudation.[1]

In special diseases of the alimentary canal the digestive juices, it need hardly be said, are abnormal. Thus in typhoid fever there is little or no pepsin secreted (Hoppe-Seyler[2]); in cancer some observers have stated there is no hydrochloric acid formed[3]; others have found the acid; probably the cases vary a good deal in this particular.[4]

Putrefaction does not occur normally in the stomach; bacteria do not flourish in an acid medium; but in various conditions in which the normal acid is absent, or masked by excess of alkaline transudation, fermentations set up by bacteria, sarcinæ, and other fungi may occur. Thus in certain cases alcoholic fermentation may take place; more frequently the lactic and butyric fermentations are set up. In these and other changes of a similar kind certain of the products are gaseous (carbonic acid, hydrogen, marsh gas) and distend the stomach, passing off ultimately in eructations. The gases are in these cases mixed with air, of which a small quantity is always swallowed with the food. On the next page are some analyses of gases from the stomach; the numbers are percentages of volume.

Gastric juice in invertebrates.—The only research I have been able to find in connection with this subject is that of Stamati[5] on the gastric juice of the crayfish. This was obtained by means of a fistula, and was found to be yellowish and alkaline; it forms peptones from proteids, sugar from starch, and emulsifies fats, liberating fatty acids. Its action thus resembles that of the pancreatic juice of vertebrates.

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[1] A most important paper on this subject is one by Leube, *Deutsch. Arch. f. klin. Med.* xxiii.

[2] *Physiol. Chem.* p. 242.

[3] v. d. Velden; Kredel, *Zeit. f. klin. Med.* vii. 592; Riegel, *Deutsch. Arch. f. klin. Med.* xxxvi. 100.

[4] H. Köster, *Maly's Jahresb.* xv. 287; *see* also Maly, *Maly's Jahresb.* xiv. 290, footnote.

[5] *Compt. rend. soc. biol.* (2) v. 16.

Gases	Stomach gases removed from human corpses[1]		Removed from stomach of a dog[1]		Gases from human beings collected from eructations[2]	
			Meat diet	Vegetable diet		
	I	II	I	II	I	II
z . . .	20·79	33·83	25·2	32·9	17·40	20·57
. . .	6·71	27·58	—	—	21·52	20·57
. . .	72·50	38·22	68·7	66·3	46·44	41·38
. . .	—	0·37	6·1	0·8	11·91	6·52
4 . . .	—	—	—	—	2·71	10·75

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[1] Planer, *Sitzungsb. Wien. Akad.* xlii.

[2] Ewald and Rupstein, *Arch. f. Anat. u. Physiol.* 1874, p. 217.

CHAPTER XXXI

DIGESTION IN THE INTESTINES

THE greyish, acid, pap-like material that leaves the stomach by the pyloric orifice is called chyme; it enters the small intestine, and is slowly propelled along it, and subsequently through the large intestine, by the peristalsis of the muscular coats. The chyme excites the pouring out of the secretions from the pancreas, the liver, and the intestinal glands. These juices are all alkaline; the acidity of the chyme is neutralised and the activity of the pepsin which left the stomach is brought to an end. When the bile meets the chyme the turbidity of the latter is increased, owing to the precipitation of certain proteids; an alkaline juice like the bile would naturally precipitate any acid-albumin, but this is not its only action. The bile-salts form with the undigested albumin, and also with the albumoses (not with true peptone), a precipitate independent of the reaction. It has been surmised that this conversion of chyme into a resinous, viscid mass is to hinder somewhat its progress through the intestine; it clings to the intestinal wall, thus allowing absorption to take place.

The intestinal contents become alkaline about ten or twelve inches from the pylorus, and then pancreatic digestion begins. This secretion continues the work commenced in the mouth and stomach, and also acts on fats; the succus entericus and bile are of minor importance. Putrefactive organisms abound and produce substances to be described later. The reaction of the intestinal contents remains alkaline until the large intestine is reached; fermentative processes have by this time produced sufficient lactic, butyric, and similar acids to more than neutralise the alkalinity of the juices. This acidity stops the digestive action of the pancreatic juice.

As the contents pass along the intestine, soluble matters are produced and then absorbed, and thus the amount of chyme is gradually diminishing. In such animals as the dog, where digestion is active and the nature of the food almost entirely digestible, the intestine will be almost empty six to nine hours after a full meal. The amount of undigested residue, which ultimately forms the fæces, is much larger in those animals, like herbivora, whose food contains a quantity of indigestible material like cellulose.

The surface of the small intestine is increased for the purposes of absorption by the folds of mucous membrane called the *valvulæ conniventes*, and also by the smaller projections called *villi.* Though these are absent from the large intestine, absorption particularly of water goes on there, and the contents thus become of more solid consistency as they approach the rectum. The length and capacity of the intestines vary much in different animals, and are related to the bulk of food these animals are in the habit of ingesting. The length of the intestines is thus much greater in herbivora than in carnivora. The horse is an exception to this rule, but the immense capacity of its intestines makes up for their shortness. Omnivorous animals, like man and the pig, occupy an intermediate position between carnivora and herbivora. The following table gives some particulars bearing out the above general statements :—

Animal	Ratio of length of intestines to length of body	Average capacity	Surface area of intestinal mucous membrane
Dog . .	5 : 1	8 litres	0·5 square metre
Cat . . .	4 : 1	—	—
Pig . . .	16 : 1	27 litres	3 square metres
Man . .	9 : 1	20 „	—
Horse . .	12 : 1	Stomach, 10–18 litres : intestine, 200 litres	15·5 square metres
Ox . . .	20 : 1	Stomach, 200 litres ; intestine, 80 litres	15 „ „
Sheep . .	26 : 1	—	—
Goat . .	26 : 1	—	—

CHAPTER XXXII

THE SECRETION OF THE PANCREAS

THE pancreas is a gland very similar in structure to the parotid gland. Its duct enters the duodenum close to the orifice of the bile-duct.

Such knowledge as we possess of the chemical composition of the pancreas as a whole has been given on p. 558. We are now more particularly interested in its secretion.

The older physiologists were quite ignorant of the vast importance of the pancreatic juice. Claude Bernard (1846) considered that it was instrumental in the emulsifying of fats. Bidder and Schmidt[1] were the first to make analyses of the juice, and our knowledge of its action and its ferments is due to the investigations of Heidenhain,[2] Bernstein,[3] Langley,[4] Lea,[5] and especially Kühne.[6]

The dog has been the animal from which pancreatic juice has been generally obtained, as the principal duct of the pancreas in this animal enters the intestine quite two centimetres from the orifice of the bile-duct. A cannula is inserted into this duct, brought through the abdominal wound and carefully stitched to it; in a few days the wound heals (Cl. Bernard). The animal suffers from not being able to carry on intestinal digestion properly, and in consequence the pancreatic juice in a day or two becomes very watery compared to that which is secreted at first.

The secretion of pancreatic juice begins in the dog immediately after the introduction of food into the stomach, and attains a maximum three hours later (Bernstein). A large amount of food increases both the quantity and the quality of the juice secreted; the juice, however, secreted at the commencement of digestion is always richer in solid constituents than that secreted later. The secretion of pancreatic juice is stated to be continuous in herbivorous animals (Heidenhain [7]).

There is at present nothing known concerning secreting nerves of the pancreas. During digestion the pancreas, however, like the other abdominal organs, is gorged with blood from dilatation of its vessels. Induction shocks applied to the organ itself or injections of blood or chyle stimulate the secretion (Kühne and Lea).

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[1] *Die Verdauungssäfte und der Stoffwechsel*, Mitau and Leipzig, 1852; *Ann. Chem. Pharm.* xcii. 33.

[2] *Pflüger's Archiv*, x. 557.

[3] *Sitzungsb. d. Akad. d. Wiss.* (Leipzig, 1869), p. 96.

[4] *Journ. Physiol.* iii.

[5] Kühne and Lea, *Verhandl. d. Heidelberg. naturhist. med. Vereins*, N.F. 1, Heft v. p. 445.

[6] *Arch. f. path. Anat.* xxxix. 130; *Heidelberg. Verhandlungen*, N.F. 1. Heft iv. and v.

[7] *Pflüger's Archiv*, xiv. 457.

Pancreatic juice is secreted under considerable pressure ; in the rabbit the pressure in the duct amounts to 16 to 17 mm. of mercury (Heidenhain).

The quantity secreted by the dog is about 2·5 grammes per kilogramme of body-weight in the twenty-four hours (Bidder and Schmidt). Colin obtained from the horse 175, from the cow 200 to 270, from the pig 12 to 15 grammes per hour. It has been calculated that a man secretes about 150 grammes of pancreatic juice *per diem.*

Microscopic examination of the gland-cells in different stages of activity reveals a series of changes comparable to those already described in the case of the salivary and gastric cells. Granules indicating the presence of a zymogen,[1] which is called trypsinogen (i.e. the precursor of trypsin, the most important ferment of the pancreatic juice), crowd the cells before secretion ; these are discharged during secretion, so that in an animal whose pancreas has been powerfully stimulated to secrete, as by the administration of pilocarpine, the granules are only seen at the free border of the cells (Kühne and Lea).

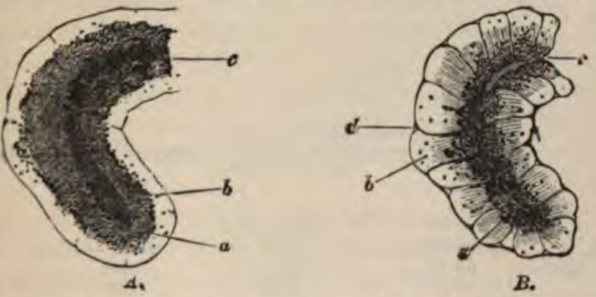

FIG. 85.—Part of an Alveolus of the Rabbit's Pancreas : A, before discharge ; B, after. (From Foster, after Kühne and Lea.)

For the investigation of the action of pancreatic juice, an artificial juice is now usually employed. A pancreas is allowed to stand at the ordinary temperature of the air for twenty-four hours ; or it may be treated with dilute acetic acid immediately ; either method converts the zymogen into the ferment. It is then minced and placed for some days or weeks under glycerin.[2] The glycerin dissolves out the ferments ; these may be precipitated from the extract by alcohol, then collected, dried at a low temperature, and preserved for future use. An artificial pancreatic juice may be then made by dissolving this in 1 per cent. sodium carbonate solution ; or a little of the glycerin extract may itself be added to the same alkaline solution ; this, however, acts more slowly because of the presence of glycerin.

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[1] Refer to p. 451. These granules are not so readily destroyed by chromic acid as those in the salivary glands and stomach.

[2] v. Wittich, *Pflüger's Archiv*, ii. 193.

COMPOSITION OF PANCREATIC JUICE

The normal secretion of the pancreas in the dog is a clear, colourless, viscid, almost syrupy fluid. It has a saltish taste and strong, alkaline reaction. The alkalinity is due to phosphates and carbonates, especially of soda. The pancreatic juice of herbivora is more watery than in the dog ; in one case of human pancreatic juice obtained by Herter the fluid was not viscid but limpid.

The following analyses (given in parts per 1000) have been made :—

	Dog		Horse	Human
	Collected on first opening the duct (Schmidt)	From a permanent fistula (Kröger).[1] Mean of 3 analyses	Hoppe-Seyler[2]	Herter[3]
Water . . .	900·76	980·44	982·53	976·0
Solids . . .	98·92	19·56	17·47	24·0
Organic matter	90·38	12·73	8·88*	18·0
Salts . . .	8·54	6·83	8·59†	6·0
KCl	—	0·93		
NaCl . . .	7·36	2·53		
Na_3PO_4 . . .	0·45	0·02		
Na_2O . . .	0·32	3·30		
$Ca_3(PO_4)_2$ . .	0·22(CaO)	0·07		
$Mg_2P_2O_7$ . .	—	0·01		
MgO . . .	0·05	0·01		

* Of this 8·6 consisted of ferments soluble in water, after precipitation by alcohol

† Containing much sodium phosphate

The organic substances present in the pancreatic ferment are :—

a. Ferments : These are the most important, both quantitatively and functionally, of all the constituents ; they are four in number :

i. Trypsin—a proteolytic ferment.
ii. Amylopsin or pancreatic diastase—an amylolytic ferment.
iii. Steapsin—a fat-splitting ferment.
iv. A milk-curdling ferment.

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[1] *Diss.* Dorpat, 1854. Quoted from Hoppe-Seyler, *Physiol. Chem.* p. 259.

[2] *Ibid.* p. 259.

[3] Quoted from McKendrick's *Physiology*, ii. 125.

b. A small amount of proteid which is coagulable by heat.

c. A mucin or mucin-like substance.[1]

d. Traces of leucine, tyrosine, xanthine, and of soaps have been described.

The ferments of the pancreatic juice.—i. *Trypsin.*—Bernard[2] and later Corvisart[3] observed that the pancreatic secretion dissolved coagulated white of egg. Kühne studied this action carefully and gave the name *trypsin* to the ferment that produces the action. Kühne prepared the ferment by means of making an aqueous extract of the pancreas at 0° C., and precipitating the proteids and ferments therefrom with alcohol. The precipitate was collected, dissolved in water, and acetic acid added till 1 per cent. of the acid was present in the solution; the precipitate so produced was again extracted with water and filtered; the filtrate was again treated in the same way, first with alcohol, then with acetic acid; the filtrate was made alkaline with soda, digested at 40° C., and filtered. The filtrate was evaporated down, and thus tyrosine crystallised out; the rest of the tyrosine, leucine, and peptone was dialysed off. Though this method gives a purer ferment than those previously adopted, yet, as Hoppe-Seyler states, the preparation cannot be regarded as absolutely pure. The substance obtained is soluble in water and in glycerin, but not in alcohol; when a solution in water is acidified faintly and heated, a heat-coagulum is formed.

Kühne[4] has more recently introduced the following method of preparing trypsin. The fresh or dried gland is first digested with 0·1 per cent. solution of salicylic acid for four hours, then with alkaline solution of thymol for twelve hours; the acid and alkaline extracts are mixed and the amount of thymol brought up to 0·5 per cent.; the amount of soda is brought up to the same percentage and the mixture is digested for six days, then cooled, and the tyrosine crystals which have formed are filtered off. It is then neutralised with acetic acid and saturated with ammonium sulphate; this precipitates the trypsin; the precipitate is collected, washed with saturated solution of ammonium sulphate, and dissolved in 0·2 per cent. soda solution; this gives a powerful digestive fluid. If one desires to get rid of ammonium sulphate, this is done by dialysis.

A conclusion which appears to be justified from these methods of preparation is that trypsin is either a proteid or a substance closely allied, or adherent, to a proteid.

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[1] In two specimens of dogs' pancreatic juice I have examined, acetic acid gave a stringy precipitate. The viscidity of the juice is evidently due to this substance, though whether it is true mucin or a nucleo-albumin I did not investigate.

[2] *Leçons*, Paris, 1855, p. 334.

[3] *Sur une fonction peu connue du pancréas*, Paris, 1858.

[4] *Centralbl. med. Wiss.* 1886, No. 8

Trypsin acts best in an alkaline medium; it also acts in a neutral medium. Stutzer[1] obtained equally good results with artificial digestion when the alkaline fluid used was 0·25, 0·5, or 1·0 per cent. of sodium carbonate. Trypsin will not act at all in an acid medium, and is destroyed by hydrochloric acid or by the acids it meets farther on in the large intestine (Langley). None passes into the urine, so probably it is entirely destroyed in the intestine. Salicylic acid, however, does not hinder the action of the ferment, so that this antiseptic can be added to artificial digestion experiments to prevent the putrefaction so generally associated with tryptic digestion[2] (Kühne). Trypsin occurs in the pancreatic secretion of new-born children, except in certain cases, and in these cases diarrhœa, generally fatal, is apt to occur.

ii. *Amylopsin.*—The diastatic action of pancreatic juice was first described by Valentin[3]; the ferment was separated in a more or less pure condition by Kröger,[4] who found it could be precipitated but not destroyed by lead acetate, though by more powerful reagents, like mineral acids, acetic acid, and alkalis, it is destroyed. Other attempts to obtain a pure product by means of extracting the gland with lime-water were made by Danilewsky,[5] and later by Cohnheim.[6] It diffuses more readily than the other ferments of the juice (v. Wittich).

It is absent in the pancreatic juice of new-born children (Korowin,[7] Zweifel[8]). Hence much starchy food is bad for very young children.

Amylopsin, like most ferments, acts best at 40° C. Like ptyalin, it converts starch into maltose. It acts better in the presence of bile than by itself.[9] Stutzer[10] found that pancreatic fluid acts better on carbohydrates when it is neutral than when alkaline; yet after the food has been subjected previously to the action of ptyalin and then of pepsin, the best results with pancreatic fluid are obtained when it is feebly alkaline.

iii. *Steapsin.*—The existence of a fat-splitting ferment in the pancreatic juice is inferred from the action of the pancreatic juice on fats.

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[1] *Zeit. physiol. Chem.* xi. 207.

[2] Other antiseptics often used are ether (Hoppe-Seyler, *Physiol. Chem.* p. 264) and arsenious acid in small quantities (Schäfer and Böhn, *Würzburg. Verhandl.* 1872, vol. iii. p. 238). I have found the latter exceedingly useful.

[3] *Lehrb. d. Physiol.* 2nd edit. 1844.

[4] *Loc. cit.*

[5] *Virchow's Archiv*, xxv. 279.

[6] *Ibid.* xxviii. 231.

[7] *Centralbl. med. Wiss.* 1873, No. 20.

[8] *Loc. cit.*

[9] Martin and Williams, *Proc. Roy. Soc.* xlv. 358. More recently (*Ibid.* xlviii. 160) these observers have shown that the bile is also favourable to the action of trypsin on proteids.

[10] *Zeit. physiol. Chem.* xii. 72.

The ferment has never been separated; it is destroyed by treating the gland with alcohol; it does not dissolve in glycerin as do the other two ferments we have described. This ferment probably exists in the secretion of the pancreas of the embryo, for free fatty acids are found in the meconium.

iv. *Milk-curdling ferment.*—The addition of pancreatic juice to milk causes clotting, but this action is seldom called into play normally, as the milk upon which the juice has to act has been already curdled by the rennin of the stomach.

THE ACTION OF PANCREATIC JUICE ON FOODS

A few simple experiments can be readily performed with artificial pancreatic juice, which will teach us the chief facts in connection with the action of that juice.

Three test-tubes are labelled A, B, and C; in each is put a few cubic centimetres of the artificial juice; the fluid in A is heated to 60° and subsequently cooled and a piece of fibrin placed in it; a piece of fibrin is also placed in B, and a few c.c. of starch solution in C. All are then put in a warm bath at 40° C.

The fibrin in A remains unaltered. This experiment illustrates the general truth that ferments are destroyed by high temperatures.

The fibrin in B undergoes fairly rapid solution; it is, however, not first swollen and then dissolved, as is the case with gastric juice, but is gradually eroded or eaten away from the edges. The products of digestion are much the same as in gastric digestion: instead of syntonin, an albuminate of the nature of alkali-albumin is formed. Albumoses and peptones (sometimes called tryptones) are present; the albumoses are more rapidly converted into peptones than in gastric digestion, and after a time some of the peptones are further decomposed, yielding leucine, tyrosine, and similar substances.

The starch in C is rapidly changed into maltose with dextrin as an intermediate product, exactly as in salivary digestion.

Another experiment which illustrates some important facts in connection with pancreatic digestion is the following: An ox pancreas, about twenty-four hours after its removal from the animal, is minced finely; the mincemeat is divided into two parts; each part is placed in a good-sized flask and a litre of 1 per cent. sodium carbonate added; the white of an egg is also added and the mixtures placed in the warm bath at 40° C. for twenty-four hours, another white of an egg being added about the middle of this period. In one flask, however, the process is rendered antiseptic either by the addition of thymol or a little 1 per cent. salicylic acid. The necks of the flasks are plugged with cotton-wool.

After twenty-four hours the fluid from each is filtered off from the undigested residue. The one to which the antiseptic was added is free or nearly free from odour; the other has an offensive fæcal odour. This second flask more correctly imitates what occurs in the intestines; an alkaline medium is the most favourable for the growth of bacteria, and bacteria thus flourish, producing indole, skatole, phenol, &c. in the intestines as well as in our flask. Various bases, amines, and ammonia are produced; leucine, tyrosine, and other acids—in fact, all the products usually derived from proteids by putrefaction. We thus have two processes occurring simultaneously in the intestine, and experiments in which putrefaction is prevented have, therefore, to be performed in order to ascertain whether the production of any particular substance is due to the pancreatic ferment, or to the accompanying putrefactive bacteria. Leucine and tyrosine will be found in the fluid in the antiseptic flask, thus showing that these products are produced by the trypsin alone; they are, however, much more abundant in the flask in which putrefaction was allowed to take place. Indole and skatole are produced by putrefaction only.

The presence of leucine and tyrosine may be demonstrated in the following ways:—

a. Take some of the fluid, add Millon's reagent, and filter off the white precipitate of mercury compounds of proteids that are formed; the filtrate is turned red on boiling; this is due to the presence of tyrosine. If the tyrosine is abundant, the filtrate is pink even before boiling.

b. Faintly acidify and boil another portion of the fluid, filter off any proteid that may be coagulated, and preserve half the filtrate for the next experiment. Evaporate the other half to a small bulk on a water-bath; mount a drop of this on a glass slide and cover. Crystals of tyrosine will be seen, and, if the evaporation has been carried on long enough, crystals of leucine also (*see* figs. 32 and 33, p. 83). Tyrosine is less soluble than leucine in water; hence it crystallises out first.

c. Take the other half of the filtrate and add excess of absolute alcohol to precipitate the peptone; filter and evaporate the filtrate to a small bulk; it becomes yellowish and sticky from the presence of leucine. Microscopic examination shows abundance of leucine balls. Tyrosine, not being soluble in alcohol, is absent. Leucine gives a well-marked xanthoproteic reaction.

d. Other methods of separating out leucine and tyrosine are given on pp. 82, 83.

Action on proteids and albuminoids.—Trypsin acts like pepsin, but with certain differences. The most striking of these differences are—

a. Trypsin acts in an alkaline, pepsin in an acid medium.

b. Trypsin acts more rapidly than pepsin, but the same series of proteoses can be detected as intermediate products in the formation of peptone.

c. An albuminate of the nature of alkali-albumin is formed in tryptic, of the nature of acid-albumin in peptic digestion.

d. Trypsin acts more powerfully than pepsin on certain albuminoids difficult of digestion, such as elastin, and waxy or albuminoid substance. It digests nuclein, which is not attacked at all by gastric juice. Keratin and chitin are, however, indigestible by both ferments.

e. Trypsin acts further than pepsin, decomposing the *hemi-peptone* into simpler products, of which the most important are leucine and tyrosine, asparaginic acid,[1] ammonia,[2] and proteinchromogen.

Proteinchromogen is a substance, originally described by Gmelin, which gives with chlorine or bromine a reddish-violet product, *proteinchrome*. These names are suggested by Stadelmann,[3] who has recently examined these substances. Nencki[4] considered that proteinchromogen is naphthylamine; a view which Krukenberg[5] and Hemala[6] showed to be untenable. Neumeister[7] has suggested the name *tryptophan* for it. Stadelmann was unable to separate proteinchromogen in a state of purity, but its bromine compound (proteinchrome) was separated by dissolving it in 90 per cent. alcohol. When dissolved in ether it shows a well-marked absorption-band in the green. Elementary analysis gave results from which the percentage composition of proteinchromogen was calculated (C, 61·02; H, 6·89; N, 13·68; S, 4·69; O, 13·71). Its reaction and composition point to its being a proteid, or a substance closely allied to a proteid.

Action on carbohydrates.—The conversion of starch into maltose is the most powerful and the most rapid of all the actions of the pancreatic juice. Kröger observed that 1 gramme of dog's pancreatic juice which contained 0·014 gramme of organic material changed 4·672 grammes of starch into sugar in half an hour at 35° C. The details of the process are exactly the same as already described for ptyalin. Not only boiled but also raw starch is affected. Glycogen is changed in the same way, more slowly than starch, but quickly in comparison to the action of saliva.[8] Cane sugar is not affected by either juice.

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[1] Radziejewski and Salkowski, *Ber. deutsch. Chem. Ges.* 1875, vol. vii. p. 1050; v. Knierem, *Zeit. Biol.* xi. 198.

[2] Hirschler, *Zeit. physiol. Chem.* x. 306; Stadelmann, *Zeit. Biol.* xxiv. 261.

[3] *Zeit. Biol.* xxvi. 491.

[4] *Ber. deutsch. chem. Ges.* vii. 1593.

[5] *Verhandl. physik. med. Ges.* Würzburg, xviii.

[6] *Chem. Unters. wiss. Med.* von C. F. W. Krukenberg, Heft ii.

[7] *Zeit. Biol.* xxvi. 324.

[8] Seegen, *Centralbl. med. Wiss.* 1876, No. 48.

Action on fats.—The action of the pancreatic juice on fats is a double one; it forms an emulsion, and it decomposes a small quantity of the fats into fatty acids and glycerin. The fatty acids unite with alkaline bases to form soaps (saponification). The chemistry of this process has been already described (p. 492). For the action of pancreatic juice on lecithin, *see* p. 531.

The chemical action of the pancreatic juice on fats cannot be demonstrated by an artificial juice made from a glycerin extract, as the steapsin is not soluble in glycerin. Either pancreatic juice from a fistula or a watery extract of pancreas must be employed. If the watery extract has partially undergone putrefaction—which it does very readily—its activity on fats is increased. The fat-splitting action of the pancreatic juice and of the organised ferments (bacteria) of the alimentary tract is identical.

If a little butter of neutral reaction be mixed with some pancreatic juice, and the mixture be put in the warm bath at 40° C. for twenty minutes, and litmus then be added, it will be turned red by the fatty acids which are liberated.

The formation of an emulsion may be studied in this way: Shake up olive oil and water together, and then allow the mixture to stand; the finely divided oil-globules will soon separate from and float on the surface of the water; but if a colloid matter, like albumin or gum, be first mixed with the water, the oil separates more slowly. A more permanent emulsion is formed by an alkaline fluid, and especially when a small amount of fatty acid is being continually liberated; the free acid combines with the alkali to form a soap which forms a thin layer outside each oil-globule. It will be seen that pancreatic fluid possesses all the necessary qualifications for forming an emulsion:—

1. It is alkaline.
2. It is viscous from the presence of proteids.
3. It has the power of liberating free acids, and thus forming a layer of soap on each oil-globule.

O. Minkowski[1] has recently found that, after extirpation of the pancreas, fats, except those in milk, are not absorbed, although splitting of the fats under the influence of bacterial agency still continues. Acidulation destroys ordinary emulsions made with an alkaline carbonate, the fat running into large drops; but with the emulsions formed in the body (milk, chyle, pancreatic emulsion) this is not the case. Kühne has suggested that in pancreatic emulsions, as in milk, each globule has an albuminous envelope which facilitates the adhesion of the fat to the absorbing cells of the intestinal wall. Whether this be so or not, it is beyond question that some peculiarity of these natural emulsions exists, which renders them easy of absorption. Minkowski's observations are also interesting as showing that fat is not absorbed to any great extent in the form of soaps. This is also borne out by K. L. Bass'[1] experiments with certain ethereal salts which resemble fats in their constitution.

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[1] *Berlin. klin. Wochensch.* 1890, No. 18.

Pathological Conditions of the Pancreatic Secretion

Very little is known on this subject. In the examination of the bilious vomit of a case of typhoid, Hoppe-Seyler[2] found the pancreatic ferment to be present. In a case of intestinal obstruction examined by the same observer, the pancreas was almost entirely replaced by a jelly-like substance having the following percentage composition: Water, 97·4; solids, 2·6; urea, 0·12; fat, 0·02; extractives, 1·40; salts, 0·57; substances insoluble in water, 0·49. The alcoholic extract contained much leucine.

Concretions are sometimes found in the ducts of the gland; these consist chiefly of calcium phosphate and carbonate.

To judge from experiments on dogs, impaired nutrition leads to a lessening of the solid constituents of the secretion. There can be but little doubt that anæmic, febrile, and debilitating conditions generally in man impair the richness and usefulness of the pancreatic juice.

Complete extirpation of the pancreas in dogs gives rise to glycosuria,[3] and Hirschfeld has shown that in diabetic patients the absorption of proteid and fat is much impaired. This is, perhaps, an indication that the cause of diabetes is in some cases disease of the pancreas. These observations are of considerable value; for, although their precise meaning has yet to be worked out, they appear to indicate that the cells of the pancreas do something else than manufacture pancreatic juice; they appear to play, in addition, an important part in the processes of general metabolism. Concomitant injuries to the nerves of the liver during the operation, as a cause of the diabetes, can be excluded, as partial extirpation of the pancreas, where there is the same risk of injuring nerves, produces no glycosuria.

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[1] *Zeit. physiol. Chem.* xiv. 416.

[2] *Physiol. Chem.* p. 269.

[3] Minkowski and v. Mering, Communication to *Internat. Physiol. Congress*, Basel, 889. *See* also Lepine, *Lyon médical*, Jan. 19 189

CHAPTER XXXIII

SUCCUS ENTERICUS

THE *succus entericus*, or intestinal juice, is the secretion of the tubular glands (crypts of Lieberkühn) which exist in the mucous membrane of the intestine. It need hardly be pointed out that it is a matter of great difficulty to obtain this secretion unmixed with other secretions, and hence there are great discrepancies in the observations that have been made on its action. Another source of fallacy is to be found in the fact that most observers have taken little or no care to exclude the presence of putrefactive bacteria. The method usually adopted of obtaining the juice is by means of a fistula, but even this is not altogether free from error, as the natural secretion is then apt to be masked by transudation from the capillaries.

Thiry's[1] method of making a fistula is to cut the intestine across in two places; the loop so cut out is still supplied with blood and nerves, as its mesentery is intact; this loop of intestine is emptied, one end is sewed up by sutures, the other stitched to the abdominal wound, and so a *cul-de-sac* from which the secretion can be collected is made. The continuity of the remainder of the intestine is restored by fastening together the upper and lower portions of the bowel from which the loop has been removed.

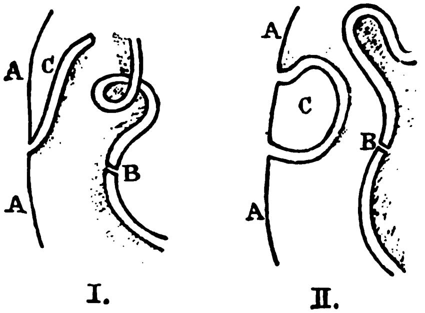

FIG. 86. Diagram of Intestinal Fistula. I, Thiry's method. II, Vella's method. A, abdominal wall; B, intestine with mesentery; C, separated loop of intestine with attached mesentery.

Vella's method resembles Thiry's, except that both ends of the loop are left free and sutured to the wound in the abdomen. Fig. 86 illustrates the two methods.

Thiry obtained a flow of secretion from the fistulous loop by electrical or mechanical stimulation; the amount thus secreted in an hour by a dog was four grammes; by calculation it was ascertained that the whole intestinal canal of the animal would secrete 360 grammes in the same time.

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[1] *Sitzungsb. Wien. Akad.* vol. l. 1864, p. 77.

The fluid was strongly alkaline, opalescent, not slimy, of a yellowish colour, and a mean specific gravity of 1010. Though the juice itself thus obtained is not viscid, it is under ordinary circumstances mixed with mucus, resulting from the breaking down of goblet cells, just as the gastric juice is mixed with gastric mucus.

The quantitative analysis of the juice gave the following results :—

Proteids	0·8013	per cent.
Other organic matters . . .	0·7337	„
Inorganic salts	0·8789	„
Total solids	2·4139	„
Water	97·5861	„

Hoppe-Seyler does not believe in the existence of a special digestive juice secreted by the intestinal glands. He attributes the action this so-called secretion has, to admixture with pancreatic juice; he looks upon the fluid obtained from a fistula merely as a transudation; and the function of the crypts of Lieberkühn is, according to him, to increase the surface of the intestine for the purposes of absorption.[1]

Though the majority of observers are opposed to this view of Hoppe-Seyler's, it must be confessed that the most trustworthy experiments on the action of the juice are of a negative character, the only positive result obtained being the existence of an inverting ferment; this inverting action might, however, be due to the mucin, and not to any special ferment.

Action of the juice on carbohydrates and fats.—*On saccharoses*: cane sugar is inverted into dextrose and levulose: the ferment which is considered the cause of this action is termed *invertin* (Paschutin,[2] Brown and Heron,[3] Seegen[4]). Milk sugar is inverted to dextrose and galactose. Maltose is inverted also, glucose being formed (Brown and Heron, Seegen). Pavy[5] made the very remarkable statement that the intestine of rabbits secreted or contained a ferment which had the opposite (dehydrating) action, and that glucose was transformed into maltose. Neither Chittenden[6] nor Ogata[7] has been able to corroborate this.

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[1] *Physiol. Chem.* p. 275.

[2] *Centralbl. med. Wiss.* 1870, Nos. 36 and 37; *Arch. f. Anat. u. Physiol.* 1871, p. 305.

[3] *Abst. Chem. Soc. Journal*, 1880, p. 903.

[4] *Pflüger's Archiv*, xl. 38.

[5] *Chem. News*, 1884.

[6] *Studies from Physiol. Lab. Yale Univ.* ii. 46.

[7] *Archiv f. Hygiene*, iii. 204.

On starch : though one observer (Eichhorst [1]) has stated that a diastatic ferment is present, the bulk of evidence is on the other side; Thiry, Leube,[2] and Schiff,[3] all found that the juice had no action on starch.

On fats : the same observers found that the juice has no action on fats.

Action on proteids.—Leube and Thiry both found that, though the juice has no action on raw meat or boiled white of egg, yet peptones are slowly formed from fibrin. Schiff found that all varieties of proteids were changed into peptone by the juice.

These results were due to the non-exclusion of putrefactive bacteria; the first experiments in which an antiseptic was employed (thymol or salicylic acid) were performed by Masloff [4] ; he found that the juice has no digestive power even on fibrin. Equally careful experiments by Wenz [5] led to the same results ; he tested the power of the juice on the various forms of albumose, and found that in no case was peptone formed from them.

We thus see that the only action we can safely attribute to the juice is its action on saccharoses. This is an action of some importance. The sugar formed in the alimentary canal by saliva or pancreatic juice is maltose ; that found in the blood after absorption is dextrose ; it would therefore seem that the change from maltose to dextrose is brought about by the intestinal juice. The juice has also an excretory function ; iodine, bromine, sulphocyanides, and lithium compounds introduced into the stomach are shortly afterwards found in the fistulous loop of the intestine ; ferrocyanide of potassium, compounds of arsenic or boric acid are not excreted by this channel (Quincke [6]).

The large intestine is chiefly concerned in absorption ; feeding by the rectum, even if the food is not artificially digested beforehand, is often followed by good results, and the question arises, whether the succus entericus there has any special action. This question has, however, received but little attention ; some have supposed that the food is absorbed without undergoing any special digestive action. v. Jaksch [7] has attempted to investigate the subject by looking for ferments in the fæces ; he found there an inverting ferment, and also a diastatic ferment. The diastatic action is not due to the action of acids on the starch, as it occurs in alkaline fæces also ; it may, however, be due to the activity of bacteria.

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[1] *Pflüger's Archiv*, iv. 570.

[2] *Centr. med. Wiss.* 1868, No. 19.

[3] *Ibid.* No. 23.

[4] *Unters. physiol. Inst. Heidelberg*, 1878.

[5] *Zeit. Biol.* xxii. 1.

[6] *Arch. f. Anat. u. Physiol.* 1868, p. 150.

[7] *Zeit. physiol. Chem.* xii. 116.

The secretion of Brunner's glands.—These glands of the duodenum are very similar to, though somewhat more complex than, the pyloric glands of the stomach. The difficulty of investigating their secretion is even greater than that experienced in the investigation of the secretion of the crypts of Lieberkühn. Schwalbe[1] obtained from the cells of Brunner's glands proteids, mucin, and a ferment. Budge[2] found that an aqueous extract of them changes starch into sugar; that it digests fibrin, but not coagulated white of egg. Grützner,[3] on the other hand, found that such an extract had no amylolytic action, but that the ferment is the same as that of the pyloric gland, namely, pepsin; while Brown and Heron[4] showed that the secretion of the glands of Brunner converts maltose into glucose more actively than any other glands of the intestine.

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[1] *Arch. f. micros. Anat.* viii. 92.

[2] *Berlin. klin. Wochensch.* 1870, No. 1.

[3] *Pflüger's Archiv*, xii. 288.

[4] *Loc. cit.*

CHAPTER XXXIV

BILE

In our consideration of the liver in Chapter XXV, we ascertained the general composition of its substance, and studied at considerable length its glycogenic function. We also noted in passing that the liver is one of the principal places for the formation of urea, uric acid, and other products of nitrogenous metabolism. This is a subject the consideration of which must be postponed till we discuss the physiology of the urinary secretion. The liver in addition to these important functions secretes bile, and it is this which we have now to take up.

The subject may be conveniently discussed under the following heads:—

1. The physiology of the secretion of bile.
2. The general composition and individual constituents of the bile.
3. The uses of bile in the intestine.
4. Certain pathological conditions, such as jaundice, gall-stones, &c.

1. THE SECRETION OF BILE

The liver is the largest gland of the body, and its structure is entirely different from that of the tubular and racemose glands we have hitherto considered. The secreting cells are massed together into small lobules or acini, which vary in diameter from $\frac{1}{24}$ to $\frac{1}{12}$ inch (one to two millimetres). These are completely isolated from one another by areolar tissue in some animals, as the pig; but in most animals, man included, they are to a great extent confluent. The cells of which the lobules are made up are of a compressed spheroidal or polyhedral form, varying in diameter from $\frac{1}{1080}$ to $\frac{1}{840}$ inch. They are granular, of a faint yellowish tinge, and contain certain quantities of glycogen and fat, the amount varying with the period of digestion. Not unfrequently two nuclei are found in one cell. The cells do not surround any lumen into which they can pour their secretion, but the minutest bile-capillaries penetrate between the cells in all directions and on all sides. The liver-cells may be considered to be packed between and around both blood-vessels and bile-vessels.[1] The walls of the blood-vessels, however, are not everywhere in contact with the liver-cells, but are separated from them in parts by cleft-like lymph spaces.

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[1] Recent investigations (*see* Lewis Jones and Shore, *Journ. Physiol.* x.) have shown that in the lower vertebrates, the liver is a compound tubular gland, but in the higher vertebrates this structure is obscured by the greatly ramified interpenetration of vessels.

The secretion of bile is influenced to a great extent by the blood supply of the liver; we will therefore first briefly refer to the circulation in that organ. The liver receives arterial blood by the hepatic artery; but this artery is a comparatively small one, and is concerned chiefly in supplying the supporting connective tissue of the organ. The chief supply of blood is venous. The portal vein is formed by the union of the veins from the intestines and spleen, enters the liver, and breaks up into capillaries after the manner of an artery; they join together and ultimately form large vessels called the hepatic veins, which carry the blood from the liver into the inferior vena cava. Certain products of digestion, especially those of a carbohydrate nature, absorbed from the alimentary canal are carried by the portal vein to the liver, and there stored up for future use. Although the capillaries of the hepatic artery probably anastomose with the portal capillaries, there can be no doubt that the bile is formed chiefly from the portal blood.

When a section of the liver is examined microscopically minute apertures may be observed between the opposed sides of adjacent liver-cells. These are the sections of the intercellular bile-canaliculi, which form a network much finer and closer than the capillary network, from the branches of which they run apart. These passages are bounded by a delicate proper wall, and open at the circumference of the acinus into the biliary ducts proper, which by uniting, ultimately form the bile-duct that leaves the liver.

To demonstrate the intercellular network, Chrzonszczewsky employed a method of natural injection. A saturated aqueous solution of sulph-indigotate of soda is introduced into the circulation of dogs and pigs by the jugular vein. The animals are killed an hour and a half afterwards, and the blood-vessels washed free from blood, or injected with gelatine stained with carmine. The bile-ducts are then seen filled with blue, and the blood-vessels with red material. If the animals be killed sooner than this, the pigment is found within the hepatic cells, thus demonstrating it was through their agency that the canals were filled.

Pflüger and Kupffer[1] have since this shown that the relation between the hepatic cells and the bile-canaliculi is even more intimate, for they have demonstrated the existence of vacuoles in the cells communicating by minute intracellular channels with the adjoining bile-canaliculi (fig. 87). It is important to notice that the bile-canaliculi are always separated by at least a portion of a cell from the nearest blood-capillaries, and that the formation of bile is no mere transudation from the blood or lymph. The liver-cells take certain materials from the lymph and elaborate the constituents of the bile, the bile-salts and the bile-pigments. There can be no doubt that these substances are formed by the hepatic cells, for they are not found in the blood nor in any other organ or tissue ; and after extirpation of the liver they do not accumulate in the blood. Even the water in the bile cannot be explained as the result of blood-pressure. Heidenhain[1] found that the pressure in the bile-duct of the dog was 15 mm. of mercury, which is about double that in the portal vein. Although pressure in the portal capillaries cannot account for the secretion, the rapidity of the flow of blood through these capillaries has an important influence, the activity of the hepatic cells depending on the amount of blood they receive in a unit of time. The secretion of bile is continuous, but it is accelerated under certain conditions ; for instance, after the ingestion of food ; and this is probably by means of a reflex mechanism. Whether it is carried out solely by means of the vaso-motor nervous system, or by means of special secretory or trophic nerves, or whether both factors are called into play, we cannot at present say. All we are acquainted with is the influence of blood-supply on the secretion ; no special trophic or secretory nerves have as yet been demonstrated to exist. Pflüger has long held that nerve-filaments terminate in hepatic cells, and though Maccallum[2] states that he also has traced minute nerve-

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[1] *Arch. mikr. Anat.* xii.

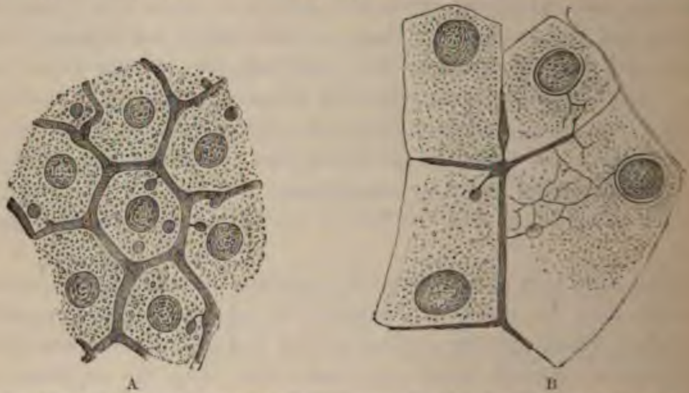

FIG. 87.—Sketches illustrating the mode of commencement of the Bile-canaliculi within the Liver-cells (Heidenhain, after Kupffer). A, rabbit's liver, injected from hepatic duct with Berlin blue. The intercellular canaliculi give off minute twigs which penetrate into the liver-cells, and there terminate in vacuole-like enlargements. B, frog's liver naturally injected with sulph-indigotate of soda. A similar appearance is obtained, but the communicating twigs are ramified.

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[1] *Hermann's Handbuch*, 1880.

[2] *Quarterly Journ. Micros. Sc.* new series, xxvii. 452.

twigs into the interior of the cells, histologists, as a rule, do not admit that these observations are free from error.

The nerves of the liver arise from the cœliac plexus and from the pneumogastric nerves (especially the left), and they enter the liver in close relation with the hepatic artery and its branches. Injury to these nerves or to their centre in the medulla produces a profound disturbance, resulting in a derangement of the glycogenic function of the liver, and the consequent appearance of sugar in the urine. It is difficult to suppose that this is wholly due to vaso-motor disturbance, since the vaso-motor nerves are distributed chiefly to the less important vessels of the liver. The portal vein, like veins generally, has a comparatively small amount of muscular tissue, and its calibre varies but little under vaso-motor influence. It would therefore appear that these nerves contain fibres exerting a trophic influence, and such an influence would no doubt have an effect not only on the glycogenic, but also on the secretory activity of the cells.

It is very necessary to distinguish carefully between the bile-secreting and the bile-discharging mechanism. When a dilute acid is applied to the orifice of the bile-duct in the intestine, a suddenly increased discharge of bile occurs, and there can be little doubt that the acid chyme which leaves the stomach has the same effect. The discharge is due to the contraction of the walls of the gall-bladder, and of the duct that leads from it. In cases of biliary fistulæ, however, the secretion of bile is found to be continuous, though not uniform ; the bile, however, does not pass into the intestine when digestion is not going on, but goes back along the cystic duct into the gall-bladder, where it is temporarily stored.

The sudden discharge of bile that occurs immediately on the arrival of food into the duodenum is not the only dose of bile that the chyme receives ; the secretion continues to flow, but more slowly, and ultimately rises again, reaching its maximum some hours afterwards. This observation has been made chiefly on animals, in whom a biliary fistula has been made, and all the bile is discharged through a cannula, collected, and carefully measured ; a few cases of biliary fistula in man have occurred after surgical operations for gall-stones, and measurements have here also been made. It must be remembered that in such cases we are not dealing with a strictly normal state of things, and this quite well accounts for the discrepancies between the statements of various observers as to when the greatest rise of bile-secretion takes place. Bernard says seven ; Bidder and Schmidt, twelve ; Kölliker and Müller, from three to five and six to eight ; Wolff,[1] from two to four and eight to sixteen; and Hoppe-Seyler,[1] five to six hours after food is taken. Copeman and Winston,[2] in a case of human biliary fistula, found a primary rise immediately on taking food; this was followed by a second rise two hours latter.

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[1] *Centralbl. med. Wiss.* 1869, No. 6.

It has already been stated that this secretion is influenced largely by the conditions of the blood-supply, and the question will be asked, how is it that the blood-supply of the liver varies, seeing that its principal vessel is not much influenced by vaso-motor phenomena? In answering such a question, we must leave out of account—because it is not proved to exist—any influence of special secretory nerves. The answer to the question is well put by Foster[3] as follows: 'When digestion is going on, all the minute arteries of the stomach, intestine, spleen, and pancreas are dilated; and general arterial pressure being somehow or other maintained, a relatively large quantity of blood rushes into the vena portæ, and the pressure in that vessel becomes much increased, though, of course, remaining lower than the general arterial pressure. Moreover, during digestion, peristaltic movements of the alimentary canal are active, and these movements, serving as aids to the circulation, help to increase the portal flow. Further the spleen . . . seems to act as a muscular pump, driving the blood onward with increased vigour along the splenic vein to the liver. So that even were the liver not connected with the central nervous system by a single nervous tie the tide of blood through the liver would ebb and flow according to the absence or presence of food in the alimentary canal.'

The second increase in the flow of bile, Bidder and Schmidt consider, is probably due to the direct effect of the digestive products carried by the blood to the liver, stimulating the liver-cells to secretory activity; this is supported by the fact that proteid food increases the quantity of bile secreted, whereas fatty food, which is absorbed not by the portal vein, but by the lacteals, has no such effect (Bidder and Schmidt, Wolff).

The chemical processes by which the constituents of the bile are formed from those of the blood are complicated and involved in obscurity. We, however, know that the biliary pigment is produced by the decomposition of hæmoglobin. Bilirubin is, in fact, identical with the substance hæmatoidin, which we have already considered in connection with the blood (p. 293). An injection of a solution of hæmoglobin into the portal vein,[4] or of substances that liberate hæmoglobin from the red blood-corpuscles, produces an increase of pigment in the bile. The statement will be found very generally made that the spleen liberates hæmoglobin from a certain number of corpuscles, and that this is carried in solution in the blood-plasma of the splenic vein to the liver, where it is seized by the cells and manufactured into bile-pigment. Anyone can easily disprove this statement for himself, as Professor Schäfer has recently done.[1] If blood be collected from two vessels, say, the splenic vein and the carotid artery, and allowed to clot, the serum will in each case be found free from hæmoglobin. If the spleen takes any part at all in the elaboration of bile-pigment, it does not proceed so far as to liberate pigment from the corpuscles; it may, however, render the pigment there more readily separable by the liver-cells.

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[1] *Physiol. Chem.* p. 285.

[2] *Journ. Physiol.* x. 213.

[3] *Text-book*, ii. 436.

[4] Tarchanoff, *Pflüger's Archiv*, ix. [illegible]

Injection of an aqueous solution of bile-salts into the blood, according to Huppert,[2] is followed by an increase of bile-salts in the bile; according to Socoloff,[3] this is not the case. This is a very fair sample of the knowledge we possess on the formation of these salts. In all probability the bile-salts (glycocholate and taurocholate of soda) when formed serve over and over again; we shall discuss this question again under the heading 'The Fate of the Biliary Constituents' (p. 687). For the present it will be sufficient to say that it is generally believed that the bile-salts in the intestine undergo breaking down, the simpler constituents so formed are absorbed, and taken to the liver again, where once more they serve for the building up of bile-salts.

To the above general facts concerning the secretion of bile a few references to important experiments on which our knowledge is based may be here briefly added.

Simultaneous ligature of the hepatic artery and portal vein abolishes the secretion (Röhrig).[4]

If the hepatic artery be ligatured the portal vein alone supports the secretion (Schiff,[5] Schmulewitsch,[6] Asp[7]).

Complete ligature of the portal vein rapidly causes death; but if the branch to one lobe be ligatured there is a slight secretion in that lobe, so that in this case the bile must be formed from arterial blood (Schmulewitsch, Asp).

If the blood of the hepatic artery is allowed to pass into the portal vein which has been ligatured on one side, secretion continues (Schiff).

Profuse loss of blood arrests the secretion.

All conditions that cause contraction of the abdominal blood-vessels diminish the secretion; so also do all conditions that cause congestion or stagnation of the blood in the vessels of the liver (Heidenhain[8]).

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[1] *Proc. Physiol. Soc.* 1890, p. 9.

[2] *Arch. d. Heilkunde*, v. 237.

[3] *Pflüger's Archiv*, iii. 598.

[4] Virchow and Hirsch, *Med. Jahresb.* vol. i. 1873, p. 143.

[5] Italian papers referred to in Hoppe-Seyler's *Physiol. Chem.* p. 281.

[6] *Ber. sächs. Akad. Wiss.* 1868.

[7] *Ibid.* 1873.

[8] *Studien des Physiol. Inst.* Breslau, Heft ii.

2. THE CHARACTERS OF BILE AND ITS CONSTITUENTS

The methods of obtaining bile are the following :—

After death it can be readily obtained from the gall-bladder. During life it can only be obtained by means of a biliary fistula ; an incision is made in the abdomen, the common bile-duct is divided, and a cannula inserted into the end in connection with the liver ; this cannula is brought through and fastened to the wound in the abdomen, which soon heals. In another method the gall-bladder is opened and stitched to the abdominal wound. The liver pours its secretion now not into the intestine, but outside the body altogether through the external opening. This operation was first performed in animals by Schwann ;[1] since then Blondlot,[2] Bidder and Schmidt, Heidenhain, Schiff, and many others have studied the secretion, and the constituents of bile by the same method.

A few cases have occurred in which by surgical interference a biliary fistula has been established in human beings ; and in some of these the secretion has been carefully studied. The most important of these cases have been recorded by Ranke,[3] v. Wittich,[4] Monro,[5] Jacobsen,[6] Yeo and Herroun,[7] Copeman and Winston,[8] and Mayo Robson.[9]

In both Ranke's and Monro's cases a liver abscess caused a communication between the lung and the gall-bladder, and the bile mixed with bronchial mucus was coughed up. In v. Wittich's and Yeo's cases the patient was suffering from serious disease ; Jacobsen says nothing regarding the health of his patient ; Copeman's case is the most interesting of the series to the physiologist, as the woman was not only in good health, but actually gained weight though all the bile was discharged externally.

The quantity of bile secreted.—The statements of different observers vary very much on this question. The quantity in the twenty-four hours secreted by a human being is put down as 652 c.c. by Ranke, 532·8 c.c. by v. Wittich, 374·5 by Yeo and Herroun, and 779·6 by Copeman and Winston, or 2·5 pints per diem in a man of 12 stone. In animals the following numbers from Bidder and Schmidt are sufficient to illustrate the great variations in different parts of the animal kingdom; the numbers given are in grammes per kilo. of body-weight in the twenty-four hours.

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[1] *Arch. f. Anat. u. Physiol.* 1844, p. 124.

[2] *Essai sur les fonctions du foie*, Paris, 1846.

[3] *Die Blutvertheilung u. d. Thätigkeitswechsel der Organe*, Leipzig, 1871 ; *Grundriss d. Physiol. d. Mensch.* 4th edit. p. 324.

[4] *Pflüger's Archiv*, iii. 781.

[5] *Cycl. Anat. und Physiol.* iii. 180.

[6] *Ber. d. deutsch. chem. Ges.* iv. 1026.

[7] *Journ. of Physiol.* v. 116.

[8] *Ibid.* x. 213.

[9] *Proc. Roy. Soc.* xlvii. 499.

	Cat	Dog	Sheep	Rabbit	Goose	Crow
Fresh bile . .	14·50	19·990	25·416	136·84	11·784	72·096
Solids . . .	0·816	0·988	1·344	2·47	0·816	5·256

A number of observations have also been made as to the influence of drugs in promoting the flow of bile; but in these experiments, as in those of stimulating nerves, it is necessary, but not always practicable, to distinguish between the bile-secreting mechanism (the liver-cells) and the bile-expelling mechanism (the contractions of gall-bladder and bile-ducts).

Mercuric chloride produces an increase of water and a diminution of the solids in the bile (Scott[1]).

Calomel, mercuric chloride, and taraxacum are not able to promote a flow of bile (Bennett, Rutherford, Gamgee).[2] These drugs, generally regarded as hepatic stimulants, probably act on the bile-expelling mechanism.

Podophyllin, rhubarb, aloes, colchicum are probably true cholagogues (Rutherford and Vignal).[3]

Various laxatives act in rabbits as cholagogues (Röhrig).[4]

The constituents of the bile.—The constituents of the bile are mucin, a mucin-like nucleo-albumin, the bile-salts proper (taurocholate and glycocholate of soda), the bile-pigments (bilirubin, biliverdin, &c.), small quantities of fats and soaps, cholesterin, lecithin, urea, and inganic salts, of which sodium chloride and the phosphates of iron, calcium, and magnesium are the most important; traces of copper are also stated to be present.

Bile is a yellowish, or reddish-brown, or dark-green, transparent fluid. The cause of the variations in its colour is due to the preponderance of the red (bilirubin) or the green pigment (biliverdin). It has a musk-like odour, a bitter-sweet taste, and a neutral or faintly alkaline reaction.

The bile removed from the gall-bladder is more concentrated than that intercepted on its way from the liver to the gall-bladder. There appear to be two reasons for this: first, during its stay in the gall-bladder a certain amount of its water is absorbed; and, secondly, there is added to it by the walls of the gall-bladder and larger ducts the substances which give it its viscidity (mucin and nucleo-albumin).[5] The specific gravity of human bile from the gall-bladder is 1026 to 1032; that from a fistula 1010 to 1011 (Jacobsen).

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[1] *Beale's Arch. of Medicine*, October 1858.

[2] *Brit. Med. Journ.* 1869, p. 411.

[3] *Ibid.* October to December 1875.

[4] *Loc. cit.*

[5] The insufficiency of these explanations is, however, well pointed out by Yeo and Herroun, *Loc. cit.* p. 120.

Quantitative composition of human bile.—The following table, taken from Yeo and Herroun's paper (the particulars regarding Copeman's case have been added), gives the mean percentage of solids in human bile as found by various observers:—

Observer	Mean percentage of solids	Origin of bile
Copeman and Winston	1·4230	Biliary fistula (healthy person)
Yeo and Herroun	1·3468	„ „ (case of cancer)
Jacobsen	2·26	„ „ (case ?)
Ranke	3·16	Broncho-biliary fistula
Trifanowski[1]	9·021	Bile collected *post mortem* (various diseases)
Gorup-Besanez[2]	13·96	Sudden death of healthy individuals
Frerichs[2]	14·04	

Here the most striking fact is the low percentage of solids in fistula-bile as compared with bladder-bile. This cannot be explained on the score of ill-health, for the percentage in the first two cases is about equal; it is only explicable partially by the fact that fistula-bile does not stay in the gall-bladder. The low percentage of solids is, as the next table shows, almost entirely due to want of bile-salts; this can be accounted for in the way first suggested by Schiff; that there is normally a bile circulation going on in the body, a large quantity of the bile-salts that pass into the intestine being reabsorbed and again secreted. Such a circulation would obviously be impossible in cases where all the bile is discharged to the exterior and so lost.

The following table gives analyses of human bile, in the 2nd and 3rd columns of fistula-bile, in the 4th column presumably of normal bile.

Constituents	Fistula bile (healthy woman. Copeman and Winston)	Fistula bile (case of cancer. Yeo and Herroun)	Normal bile (Frerichs)
Sodium glycocholate .	0·6280	0·165	9·14
Sodium taurocholate .		0·055	
Cholesterin, lecithin, fat	0·0990	0·038	1·18
Mucus . . .	0·1725	0·148	2·98
Pigment . . .	0·0725		
Inorganic salts . .	0·4510	0·878 (including extractives)	0·78
Total solids . . .	1·4230	1·284	14·08
Water (by difference) .	98·5570	98·716	85·92
	100·0000	100·000	100·00

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[1] *Pflüger's Archiv*, ix. 492.

[2] *Lehrbuch d. physiol. Chem.*, by Gorup-Besanez, 3 Auf. p. 529.

This table illustrates the fact that of the two bile-salts the glycocholate is the more abundant. Many other observers who have published analyses of human bile note the same fact. The proportion of the two bile-salts is thus given in percentages:—

	Socoloff[1]	Hoppe-Seyler[2]
Sodium glycocholate . .	4·804 . .	3·03
Sodium taurocholate . .	1·567 . .	0·87

Quantitative composition of the bile of the lower animals.—The following tables are taken from Hoppe-Seyler's work on physiological chemistry (p. 302 *et seq.*) :—

Percentage composition of dog's bile (analyses by Hoppe-Seyler):—

Constituents	Bladder bile		Freshly secreted bile	
	I	II	I	II
Mucin	0·454	0·245	0·053	0·170
Sodium taurocholate . .	11·959	12·602	3·460	3·402
Cholesterin	0·449	0·133	0·074	0·049
Lecithin	2·692	0·930	0·118	0·121
Fat	2·841	0·083	0·335	0·239
Soaps	3·155	0·104	0·127	0·110
Other organic matters insoluble in alcohol	0·973	0·274	0·442	0·543
Inorganic matters insoluble in alcohol	0·199	—	0·408	—
K_2SO_4	0·004	—	0·022	—
Na_2SO_4	0·050	—	0·046	—
NaCl	0·015	—	0·185	—
Na_2CO_3	0·005	—	0·056	—
$Ca_3(PO_4)_2$	0·080	—	0·039	—
$FePO_4$	0·017	—	0·021	—
$CaCO_3$	0·019	—	0·030	—
MgO	0·009	—	0·009	—

We see, as before, that the bile from the gall-bladder is more concentrated than that which is freshly secreted, and that this is chiefly shown in the percentage of bile-salts, which in the dog consist almost exclusively of taurocholate of soda. The small percentage of sodium chloride is due to the fact that the greater part of that salt was dissolved by the alcohol and not estimated. The amount of taurocholate present may be easily estimated from the amount of sulphur in the dry residue of the alcoholic extract, taurocholic acid being the only substance there that contains sulphur (*see* further p. 681).

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[1] *Pflüger's Archiv*, xii. 54.

[2] *Physiol. Chem.* p. 301.

Percentage composition of the bile of various animals:—

Constituents	Ox[1]	Pig[2]	Kangaroo[3]	Goose I[4]	Goose II[5]	Python[6]
Mucin and pigment	0·30	0·59	4·34	2·56	3·1	0·89
Bile-salts	8·00	8·38	7·59	14·96	16·4	8·46
Cholesterin, lecithin, and fat		2·23	1·09	0·36	0·3	0·03
Inorganic salts	1·26			2·10	2·6	0·20
Total solids	9·56	11·20	14·13	19·98	22·4	9·58
Water	90·44	88·80	85·87	80·02	77·6	90·42

The dry residue of the alcoholic extract contains the following percentages of sulphur (Bensch,[7] Strecker):—

Dog	6·21	Goat	5·20
Fox	5·96	Pig	0·33
Wolf	5·03	Hen	4·96
Bear	5·84	Pike	5·77
Ox	3·58	Cod	5·66
Calf	4·88	Perch	5·99
Sheep	5·71	Plaice	5·91

The amount of iron in the bile is important. The iron is present as a phosphate, and there can be no doubt that it is derived from hæmoglobin. The bile-pigment is formed from hæmoglobin, but is free from iron. Some of this iron is stored in the liver-cells, some discharged as phosphate in the bile. The percentage of iron in the bile is thus given by various observers:—

Observer	Human bile	Dog's bile	Ox-bile
Young[8]	0·004 to 0·010	0·016	0·003 to 0·006
Hoppe-Seyler[9]	0·0062	0·0063 to 0·0078	—
Kinckel[10]	—	0·0058	—

The amount of iron discharged in the excretions (bile and urine) is small compared with the amount of hæmoglobin destroyed to form biliary and urinary pigments. The remaining iron is stored in the liver-cells as a compound with nuclein and proteids. The compound so formed may occur in the form of pigment-granules in the cells, or as a diffuse, colourless, soluble substance. According to Delépine these iron compounds are once more elaborated into new hæmoglobin for the young red corpuscles. This he describes as the ferrogenic function of the liver (*see* p. 552).

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[1] Berzelius, *Lehrbuch*, Dresden, 1831.

[2] Gundlach and Strecker, *Ann. Chem. Pharm.* lxii. 205.

[3] Schlossberger, *Ibid.* cx. 244.

[4] Marsson, *Arch. d. Pharm.* lviii. 138.

[5] Otto, *Ann. Chem. Pharm.* clix. 189.

[6] Vogtenberger and Schlossberger, *Ibid.* cviii. 66.

[7] *Ibid.* lxv. 215.

[8] *Journ. Anat. and Physiol.* (2), vii. 158.

[9] *Loc. cit.*

[10] *Pflüger's Archiv*, xiv. 353.

The gases of the bile have been examined by Pflüger,[1] Boguljobow,[2] and Noël.[3] Oxygen is absent or present in the merest traces; the most important gas is carbonic acid; it, however, diminishes during the stay of bile in the gall-bladder. The carbonic acid is present in two conditions, one part being free and removable merely by placing the bile in a vacuum; the other part is more firmly combined, and requires the addition of some other acid, such as phosphoric acid, to drive it off. The numbers given vary very much, the free carbonic acid from 5 to 17 vols. per cent., the combined carbonic acid from 0·6 to 62 per cent. Small quantities of nitrogen are found in addition (*see* also p. 392).

Bile-mucin

Landwehr[4] was the first to point out that the slimy substance in bile is not a compound of a proteid with a carbohydrate radicle as are the true mucins. He considered it to be a mixture of serum-globulin with the bile-salts. An examination of his analytical results shows that there is some difficulty in accepting this view; and, although a mixture of sodium glycocholate with serum-globulin has the physical characters of bile-mucin, a mixture of globulin with bile deprived of its so-called mucin does not produce the characteristic viscidity of normal bile.

This mucinoid substance can be precipitated from bile by means of acetic acid or by excess of alcohol. It is, unlike true mucin, slightly soluble in excess of acetic acid. Paijkull[5] has under Hammarsten's superintendence prepared the substance by precipitation with alcohol. He found, like Landwehr, that this substance is not true mucin, though it may contain small quantities of true mucin apparently derived from the walls of the gall-bladder. On gastric digestion it yields an insoluble residue of nuclein. The so-called mucin of bile is therefore chiefly a nucleo-albumin. Whether it is derived wholly from the walls of the ducts and gall-bladder, or is partly formed by the liver-cells, merits a fresh investigation.

The Bile-salts

The bile contains the sodium salts of complex amido-acids called the bile-acids. The two acids most frequently found are glycocholic and taurocholic acids.

Glycocholic acid ($C_{26}H_{43}NO_6$) is especially abundant in the bile of herbivora and in man; its amount is increased by a vegetable diet. By the action of dilute acids and alkalis, and also in the intestine, it takes up water and splits into glycocine or amido-acetic acid and cholalic acid.

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[1] *Pflüger's Archiv*, ii. 173.

[2] *Centralbl. med. Wiss.* 1869, No. 42.

[3] *Etude générale sur les variations des gaz du sang.* Thèse. Paris, 1876.

[4] *Zeit. physiol. Chem.* viii. 114.

[5] *Ibid.* xii. 196.

$$\underset{\text{[glycocholic acid]}}{C_{26}H_{43}NO_6} + H_2O = \underset{\text{[glycocine]}}{C_2H_5NO_2} + \underset{\text{[cholalic acid]}}{C_{24}H_{40}O_5}$$

The glycocholate of soda has the formula $C_{26}H_{43}NaNO_6$.

Taurocholic acid ($C_{26}H_{45}NO_7S$) is especially abundant in the bile of carnivora. By the action of hydrolysing agents and in the intestine it splits into taurine and cholalic acid.

$$\underset{\text{[taurocholic acid]}}{C_{26}H_{45}NO_7S} + H_2O = \underset{\text{[taurine]}}{C_2H_7NO_3N} + \underset{\text{[cholalic acid]}}{C_{24}H_{40}O_5}$$

The taurocholate of soda has the formula $C_{26}H_{44}NaNO_7S$.

Cholalic acid ($C_{25}H_{40}O_5$) is derived from the decomposition of the bile-acids. Its constitution is at present unknown. *Choleic acid* ($C_{25}H_{42}O_4$) has been separated in small quantities from ox-bile; and *fellic acid* ($C_{23}H_{40}O_4$) from human bile. It is admixture with fellic acid that renders the cholalic acid of human bile apparently different from that obtained from other sources. *Hyo-cholalic acid* ($C_{25}H_{40}O_4$) replaces cholalic acid in *hyo-glycocholic* and *hyo-taurocholic acid*, the acids of pig's bile. In the bile of the goose, cholalic acid is replaced by *cheno-cholalic acid* ($C_{27}H_{44}O_4$). Further particulars concerning the bile-acids will be found on pp. 86 to 88.

The bile-acids may be prepared from bile by the following methods:—Evaporate ox-bile to a thick syrup, stirring it frequently with a glass rod; digest this with cold absolute alcohol; this leaves the pigment, mucin, and part of the mineral salts undissolved; boil the extract with animal charcoal to completely decolourise it, and filter. Another method consists in rubbing up bile with animal charcoal into a paste; this is dried on the water-bath, and extracted with absolute alcohol, and the extract filtered.

The extract having been made, the alcohol is distilled off, the residue dissolved in a little absolute alcohol, and ether added till it becomes turbid. In a few hours or days a whitish semi-crystalline mass is deposited. This is Plattner's crystallised bile, and consists of a mixture of glycocholate and taurocholate of soda. This is dissolved in water, a little ether is added, and then dilute sulphuric acid; stir well, and glycocholic acid crystallises out in shining needles, the taurocholic acid remaining in solution; the crystals may be collected on a filter, redissolved in dilute spirit, and precipitated with excess of ether.

Another method is as follows:—Dissolve Plattner's crystals in water; add neutral lead acetate, and lead glycocholate is precipitated; collect this on a filter, wash, dissolve in hot alcohol, and remove the lead by a stream of sulphuretted hydrogen; filter off the lead sulphide; add water carefully to the filtrate, and crystals of glycocholic acid will be precipitated. To the previous filtrate from the glycocholate of lead add basic acetate of lead and ammonia; taurocholate of lead is precipitated, from which taurocholic acid may be prepared, as glycocholic acid is from the glycocholate of lead.

Hüfner's method, as modified by Marshall,[1] for obtaining glycocholic acid is the following: A little hydrochloric acid is added to fresh bile, the mixture shaken, and the mucinoid material so precipitated filtered off. Ethyl ether and hydrochloric acid are added to the filtrate; the proportion of filtrate : acid : ether = 100 : 5 : 30. The mixture is shaken and allowed to remain some hours, when crystals form, which are then collected on a filter, washed with water holding hydrochloric acid and ether in solution, and dried in the air. By recrystallisation they are obtained perfectly colourless.

In the preparation of taurocholic acid one would preferably use dog's bile. To determine its amount quantitatively take the dried alcoholic extract of a known quantity of bile; evaporate it to dryness on the water-bath with fuming nitric acid; the sulphur is thus converted into sulphuric acid; digest the residue with water, and determine the sulphuric acid by titration with alkali (p. 16): 98 parts $H_2SO_4 = 32$ sulphur; and 1 part sulphur = 16·8 taurocholic acid.

To prepare cholalic acid boil bile with caustic potash for twelve to twenty-four hours; then precipitate with hydrochloric acid; wash the precipitate with water, and dissolve it in caustic soda containing a little ether; render this acid with hydrochloric acid, and crystals form after a time; decant, cover the residue with ether; drain off the ether in half an hour, and dissolve the deposit in boiling alcohol; to this solution add a little water till a permanent precipitate appears; tetrahedric crystals of cholalic acid soon form.

To prepare glycocine, glycocholic acid is boiled for a long time with strong hydrochloric acid; the firm resin (bile-resin) that is formed consists of cholalic acid and dyslysin; this is filtered off, and the filtrate yields on evaporation hydrochloride of glycocine ($C_2H_5NO_2.HCl$). This is dissolved in water, treated with lead hydrate, filtered, and the soluble lead compound of glycocine in the filtrate decomposed by a stream of sulphuretted hydrogen. The lead sulphide is filtered off, and the filtrate on concentration yields crystals of glycocine, which may be purified by recrystallisation.

Taurine is best obtained from dog's bile; this is concentrated and then boiled several hours with hydrochloric acid; the bile-resin is filtered off, and with it some sodium chloride which has crystallised out. Evaporate the filtrate to dryness, and digest the residue with alcohol, to remove the glycocine hydrochloride if any is present. The residue insoluble in alcohol is extracted with boiling water, and the extract left to crystallise; more sodium chloride separates, the taurine remaining in solution. Decant off the liquid, and add to it four or five times its volume of boiling alcohol; this dissolves the taurine, which separates in prismatic crystals as the liquid cools. To purify it, taurine may be redissolved in water, and recrystallised by the addition of alcohol.

For the methods of obtaining the rarer forms of bile-acids the original memoirs (*see* footnotes, p. 88) must be consulted.

Pettenkofer's test.—The following reaction is given by bile, and by the bile-acids, and is apparently due to the presence of cholalic acid. Spread a drop of bile in a thin film on a porcelain capsule, and mix with it a drop of strong solution of cane sugar and a drop of strong sulphuric acid, and if necessary warm. A deep purplish-red colour appears.

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[1] *Zeit. physiol. Chem.* xi. 233.

This should be called the *furfur-aldehyde reaction*, as it is this substance formed from the sugar and acid which gives the colour with cholalic acid. It is unfortunately not distinctive of the bile-acids, being also given by numerous other organic substances. None, however, except α-naphthol give it so readily as the bile-acids; and the spectroscopic appearances are different in many instances; the colour produced by bile shows one band between D and E and another at F. A third fainter band near the D line, which fades as the reaction becomes fully marked, is described by MacMunn (*see* fig. 88, spectrum 6).

The Bile-pigments

The two principal pigments of the bile are named bilirubin (formerly known as cholepyrrhin, biliphæin, or bilifulvin) and biliverdin. Bile which contains chiefly bilirubin (such as dog's bile) is of a golden or orange yellow colour, while the bile of many herbivora, which contains chiefly biliverdin, is either green or bluish green. Human bile is generally described as containing chiefly bilirubin, but in Copeman and Winston's case, biliverdin was present in excess.

These pigments are undoubtedly formed from hæmoglobin; injection of hæmoglobin into the portal vein increases the bile-pigments. Bilirubin is identical with the substance called hæmatoidin, crystals of which form in old extravasations of blood (*see* p. 293). The above bile-pigments are free from iron, and show no absorption-bands when examined spectroscopically; but there is a strong absorption of the violet end of the spectrum in the case of bilirubin, while in that of biliverdin some of the red is absorbed.

Bilirubin has the formula $C_{16}H_{18}N_2O_3$ (Städeler,[1] Maly[2]), $C_9H_9NO_2$ (Thudichum[3]); the first is the one usually accepted. It has been prepared by dissolving it out from the bile by means of chloroform after acidulating,[4] and from the gall-stones of men and oxen by Städeler and others. It sometimes occurs in a crystalline form in the gall-bladder. It is insoluble in water, slightly soluble in alcohol and ether, readily soluble in chloroform, benzene, acids, and alkalis. Hoppe-Seyler[5] recommends the following method of preparing it. Bile is diluted with water and precipitated by milk of lime; this carries down the pigment; a stream of carbonic acid is passed through the mixture till no more precipitate forms; the precipitate is collected, suspended in water, treated with hydrochloric acid, and extracted with chloroform. From this extract the pigment is precipitated by alcohol

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[1] *Vierteljahrschr. d. Zürich. naturforsch. Ges.* viii. 1.

[2] *Sitzungsber. Wien. Akad.* lvii. and lxx.

[3] *Journ. prakt. Chem.* civ. 18

[4] Valentiner, *Gunsburg. Zeitsch.* 1858.

[5] *Physiol. Chem.* p. 294.

When bilirubin is treated with oxidising agents a series of coloured products are successively formed. This constitutes Gmelin's test for the bile-pigments. If a drop of bile be spread in a thin film on a porcelain plate, and a drop of nitric acid containing nitrous acid in solution be placed in the centre of it, the drop of acid becomes surrounded by rings of colours, green, blue, violet, red, and yellow. The green colour is the first stage, the yellow the last stage in oxidation. The green pigment is biliverdin ; the blue or violet product is called bilicyanin[1] ; its composition is unknown ; it shows certain absorption-bands. The red product has also not been further investigated. The end or yellow product was called choletelin by Maly,[2] whose formula for it is $C_{16}H_{18}N_2O_6$. It is soluble in water, alcohol, acids, and alkalis, and is amorphous. MacMunn[3] describes the spectroscopic changes that occur as follows : As the blue colour appears a broad shading composed of two bands appears at D, then a black band close to F. The two bands first mentioned are separated by a narrow interval in which the D line is seen (fig. 88, spectrum 1). As the colour changes progress the band after D fades away, then that before D ; and when the yellow stage is reached one band, that at F, is alone visible (fig. 88, spectrum 2).

Biliverdin has the formula $C_8H_9NO_2$ (Thudichum). It may occur as such in the bile ; it may be formed by simply exposing red bile to the oxidising action of the atmosphere ; or it may be formed, as in Gmelin's test, by the more vigorous oxidation produced by fuming nitric acid. It gives the remaining colours of Gmelin's test quite well.

Maly obtained biliverdin by the action of acetic acid or mono-chloracetic acid on bilirubin. It differs from bilirubin considerably in its solubilities, being soluble in alcohol, insoluble in chloroform and in water, almost insoluble in ether. It can be precipitated from bile by means of hydrochloric acid. It has never been obtained in a crystalline form.

Haycraft and Scofield[4] have recently shown that not only may the bile-pigment undergo changes of an oxidative nature, but that reduction processes may occur also ; for instance, placing the positive pole of a battery in bile, and then completing the circuit, will cause a series of colour-changes to occur, indicating oxidation ; if, now, the negative pole be substituted for this the reverse series of colour-changes occurs, indicating reduction. They also show that under certain other circumstances, especially in the presence of putrefactive organisms, reduction may occur in the bile.

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[1] Heynsius and Campbell, *Pflüger's Archiv*, v. 497.

[2] *Sitz. Wien. Akad.* lix. Abth. ii.

[3] *Clinical Chemistry of Urine*, London, 1889, p. 170.

[4] *Zeit. physiol. Chem.* xiv. 173.

Hydro-bilirubin.—If a solution of bilirubin or biliverdin in dilute alkali be treated with sodium amalgam, or allowed to putrefy, a rose-red or brown-red pigment is formed which is slightly soluble in water, easily soluble in alcohol, ether, chloroform, salt solutions, or alkaline fluids. Maly[1] investigated this substance, and gave it the name of hydro-bilirubin, and assigned to it the formula $C_{32}H_{44}N_4O_7$; it thus contains less hydrogen, and rather more oxygen, than bilirubin.

Its spectroscopic appearances are as follows: A dark band between *b* and F, and a fainter band in the region of the D line (fig. 88, spectrum 3).

The ammoniacal solution of this pigment gives on the addition of zinc-chloride a well-marked green fluorescence, and then shows three bands instead of two (fig. 88, spectrum 4).

The interest of this substance arises from the fact that many physiologists believe it is identical with the substance called *stercobilin* by Vaulair and Masius,[2] which is the colouring matter of the fæces, and according to some also with *urobilin*, the chief pigment of the urine. We shall see, when discussing those pigments, that hydro-bilirubin is not absolutely identical with either. MacMunn[3] and Disque[4] both regard hydro-bilirubin as an impure product.

Bilifuscin ($C_{16}H_{20}N_2O_4$) is a pigment which has been obtained from brown gall-stones. The gall-stones are powdered and thoroughly extracted with a mixture of ether and alcohol to remove the cholesterin, then with dilute hydrochloric acid to remove calcium salts; the acid is washed away with hot water and the residue shaken with alcohol. On distilling off the alcohol from the extract, a reddish-brown amorphous pigment is left, which is bilifuscin. It is insoluble in water, chloroform, or ether; soluble in alcohol. It shows no absorption bands. It does not give Gmelin's test (Städeler).

Biliprasin ($C_{16}H_{22}N_2O_6$) is the name given by Städeler to a green pigment which he separated from gall-stones. Maly[5] considers it is identical with biliverdin.

Bilihumin is the humous-like residue left after extracting gall-stones with water, alcohol, ether, chloroform, and dilute acid successively (Städeler). It is probably an impure substance.

Cholohæmatin.—This pigment occurs in the bile of the ox and sheep, and gives a three-banded spectrum (fig. 88, spectrum 5). An ethereal extract of the residue—obtained by agitating the acidulated bile with chloroform and evaporating this—is evaporated, and the residue again taken up with chloroform, which is washed in a separating funnel with water. On evaporating the chloroform a dark green pigment with a musky smell is left. It is considered by MacMunn,[6] who first described it, to be a derivative of hæmatin, probably an intermediate stage in the formation of biliverdin.

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[1] *Centralbl. med. Wiss.* 1871, No. 54.

[2] *Ibid.* No. 24.

[3] *Loc. cit.* p. 107.

[4] *Zeit. physiol. Chem.* ii. 259.

[5] *Ann. Chem. Pharm.* clxxv. 76.

[6] *Journ. of Physiol.* vi. 22.

Hæmoglobin itself and a substance like methæmoglobin have been described in the bile of animals killed by freezing, or after the injection of aniline, toluidine, and other substances that destroy the red blood-corpuscles (Wertheimer and Meyer[1]).

Biliary urobilin is a substance like urobilin, which has been sometimes found by MacMunn[2] in the bile of man, pig, ox, and sheep. The bile was treated with alcohol and acetic acid, and filtered; the filtrate was diluted with water and agitated with chloroform. The chloroform became orange; this extract was evaporated on the water-bath, and the pigment extracted from the residue with rectified spirit. This solution showed two bands very like those of hydrobilirubin. With ammonia and zinc chloride it gave a red, which on exposure to the air became a green fluorescence, and by further oxidation it was made to resemble choletelin. The probable origin of this pigment in the bile is as

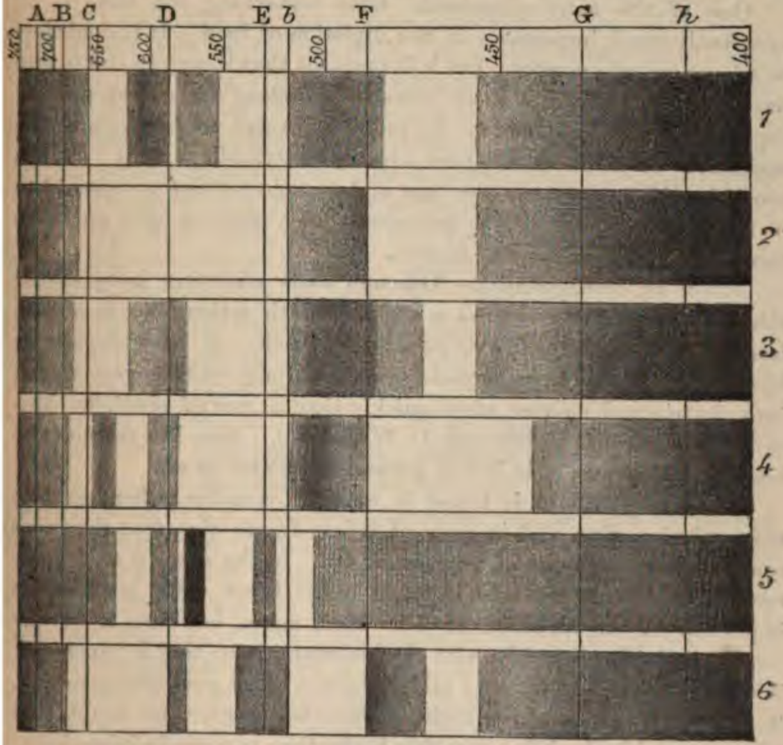

FIG. 88.—Spectrum 1 represents the bilicyanin stage of Gmelin's reaction; spectrum 2 represents the final (choletelin) stage of Gmelin's reaction; spectrum 3 is the absorption-spectrum of hydrobilirubin; spectrum 4, the same after treatment with zinc-chloride and ammonia; spectrum 5 is the absorption-spectrum of cholo-hæmatin; spectrum 6 is the absorption-spectrum of the colour formed in Pettenkofer's reaction. The faint band near D fades as the colour is developed (after MacMunn).

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[1] *Compt. rend.* cviii. 357.

[2] *Proc. Roy. Soc.* No. 208, 1880; *Journ. of Physiol.* x. 108.

follows: The bile is poured into the intestine; the pigment in the intestine is changed into a substance like hydro-bilirubin; this is absorbed and carried back by the portal circulation to the liver, and then excreted in the bile.

3. THE USES AND FATE OF BILE IN THE INTESTINE

One of the most remarkable facts concerning the bile is its apparently small importance in the digestion of food. It is doubtless to a large extent excretory, but it is probable that it may in the future be found that bile is a more valuable digestant than is at present supposed. It has no action on proteids, except to precipitate the undigested albumin, as has been already described (p. 652). It has probably slight actions on the fats and carbohydrates, but appears to be rather a coadjutor to the pancreatic juice than to have an independent digestive activity of its own.

Action on carbohydrates.—Although some observers have stated that the bile of herbivora has a slight diastatic action, bile, as a rule, has no power whatever in this direction by itself. If, however, bile or bile-salts be added to pancreatic juice, that juice will convert starch into dextrin and maltose more quickly than a control specimen containing no bile (S. Martin and D. Williams[1]). How bile favours the action of pancreatic juice it is at present impossible to say.

Action of fats.—It is found in cases of jaundice, when no bile enters the intestine, and in cases of biliary fistula also, that the fæces contain a large amount of undigested fat. In the dog, 40 to 50 per cent. of the fat in the food is found in the fæces. Bile is therefore important in the digestion of fat. Here again, however, it is the combined action of the bile, with the pancreatic juice, that is important. Although the bile is by some said to have a slight emulsifying action, it is if present at all very slight. There is, however, no doubt that pancreatic juice *plus* bile act on fats better than pancreatic juice alone.[2]

Bile is said also to aid in the absorption of fats by lubricating the mucous membrane of the bowel. If an animal membrane, such as a piece of bladder, or even a filter paper, be moistened with bile, fat will pass through it under less pressure than if they are moistened with water.[3] It is a little dangerous to draw positive conclusions from such an experiment as this. We shall see that absorption is not simply a matter of diffusion or filtration, and is a very different matter from what occurs in dead membranes; and there is perhaps no substance in which the living activity of the cells is so much needed for absorption as fat.

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[1] *Proc. Roy. Soc.* xlv. 358. More recently these observers have shown that bile also favours the action of pancreatic juice on proteids (*Ibid.* xlviii. 160).

[2] A recent paper on this subject is one by A. Dastre, *Compt. rend. Soc. biol.* 1887, p. 782.

[3] v. Wistinghausen, *Diss.* Dorpat, 1851; J. Steiner, *Arch. f. Anat. u. Physiol.* 1873, p. 137; 1874, p. 286.

Bile as a laxative and an antiseptic.—The fæces in animals or human beings who suffer from jaundice or a biliary fistula are extremely hard, and have an intense putrescent odour. Administration of bile relieves this condition; it is also known that a large increase in the flow of bile has a purgative effect as in bilious diarrhœa. The bile itself is readily putrescible, and the power it has of diminishing putrescence in the fæces is due chiefly to the fact that by increasing peristalsis it hastens the passage of putrescible matters through the bowel.[1] Copeman and Winston[2] performed a number of cultivation experiments with bacteria of different kinds, and found that, though bile is able to a small extent to control putrefactive changes, the bacteria grew almost as readily in the tubes to which bile had been added as in those to which no bile had been added. Limbourg[3] made similar experiments, and estimated certain products of putrefaction (amido-acids and ammonia) in artificial pancreatic digestions with and without the addition of bile-salts. In the specimens where the bile-salt was present, these products were somewhat lessened.

The fate of the constituents of the bile.—We have seen that fistula-bile is poor in solids as compared with normal bile, and this is explained on the grounds that the normal bile-circulation is not occurring, and hence the liver cannot excrete what it does not receive back from the intestine. Schiff[4] was the first to show that if the bile be led back into the duodenum, or even if the animal be fed on bile, the percentage of solids in the bile secreted is at once raised. It is on these experiments that the theory of a bile-circulation is chiefly founded. The bile-circulation relates, however, chiefly, if not entirely, to the bile-salts; they are found but sparingly in the fæces; they are only represented to a slight extent in the urine; hence it is calculated that seven-eighths of them are reabsorbed from the intestine, especially the large intestine. This is by no means the least curious of the phenomena of bile-secretion. The bile is a most elaborate secretion; it is poured into the intestine, and finds apparently little to do; it is split into simpler constituents, which then hurry back by the portal vessels to the liver again, when once more they unite to form bile-salts. It is stated that of the two bile-salts, the taurocholate is the more easily decomposed. Small quantities of cholalic acid, taurine, and glycocine are found in the fæces; some of the taurine is absorbed and excreted as tauro-carbamic acid in the urine (p. 85). Some of the glycocine may be absorbed and excreted as urea (Salkowski); but the greater part of all these constituents are apparently taken back to the liver to form bile-salts over again. The cholesterin and mucus of the bile are found in the fæces; the pigment is changed into stercobilin, a substance like hydro-bilirubin, but a little different from it.

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[1] McKendrick, *Physiology*, ii. 122.

[2] *Journ. Physiol.* x. 213.

[3] *Zeit. physiol. Chem.* xiii. 196.

[4] *Pflüger's Archiv*, iii. 598.

4. ABNORMAL AND PATHOLOGICAL CONDITIONS IN BILE-FORMATION.

Effect of poisons and diseases.—Many poisons, especially metallic ones, are excreted by the liver, particularly antimony, arsenic, copper, lead, and mercury (Orfila and others). Sodium indigo-sulphate after injection into the circulation soon appears both in the bile and the urine (Diakonow [1]). Iodine, grape sugar, oil of turpentine are found in the bile after injection into the circulation (Bernard). Large quantities of water similarly injected leads to the appearance of albumin, both in bile and urine (Mosler [2]).

In uræmia, the quantity of urea (which is present in mere traces in healthy bile) in the bile is increased.

In cholera, the bile also contains more urea than normal, and, like the blood, is very concentrated.

In febrile conditions generally, the amount of bile, like that of saliva and gastric juice, is diminished.

In fatty and in amyloid degeneration of the liver, the total percentage of solids, and especially of bile-salts, in the bile is greatly lessened (Ritter,[3] Hoppe-Seyler [4]).

In acute yellow atrophy of the liver, the bile, like the blood and the urine, contains leucine and tyrosine.

In typhoid fever, the bile is after death found to be sometimes acid. This may arise from decomposition of the lecithin in the bile, or from diffusion of acids from the intestine into the gall-bladder after death; leucine and tyrosine are also stated to have been found,[5] but these may arise from putrefaction after death.

Jaundice and cholæmia.—The small pressure of the bile in the bile-ducts accounts for the fact that a very slight obstruction will prevent the bile from entering the intestine; the fæces are thus almost white (clay-coloured). Bile, however, continues to be secreted, and is absorbed by the lymphatics, and entering into the circulation stains the skin and mucous membranes yellow, and passes into the urine; in the urine bile-pigment may be easily recognised by Gmelin's test; bile-acids are more difficult to discover, and seem to be often absent in the urine of such cases. Non-obstructive jaundice and cholæmia are described in Chapter XVI (p. 311).

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[1] Hoppe-Seyler's *Med. chem. Unters.* ii. p. 245.

[2] Mosler, *Ueber den Uebergang von Stoffen aus dem Blute in die Galle,* Giessen, 1857. *See* also Heidenhain, *Studien des Physiol. Inst.* Breslau, 1863.

[3] *Journ. de l'anat. et de physiol.* 1872, p. 181.

[4] *Physiol. Chem.* p. 518.

[5] Frerichs, *Wien. med. Wochensch.* 1851, p. 30.

Cholesteræmia.— Flint[1] considers that the separation of cholesterin by the bile is essential for the maintenance of the healthy activity of the nervous system, and that derangements of this secretion lead to nervous symptoms, which he designates by the name *cholesteræmia.* These observations require fuller investigation before they can be accepted.

Gall-stones.—These are concretions that may occur in the biliary passages, or more frequently in the gall-bladder. They consist chiefly of cholesterin, with a smaller amount of calcium carbonate. They may, or may not, be infiltrated with bile-pigment. v. Planta and Kekule[2] analysed some gall-stones which contained 90 per cent. of dry cholesterin. In some cases, however, the most important constituent is bile-pigment. Thudichum[3] found that the bile-pigment is chiefly bilirubin, not, however, free bilirubin, but a calcium compound of the pigment called bilirubin-calcium. In some gall-stones of this nature Maly[4] found 28 to 45 per cent., and Phipson[4] 61 per cent. of bilirubin. Städeler found in addition biliverdin, bilifuscin, biliprasin, and bilihumin. The nucleus or central portion of a gall-stone appears to be chiefly mucus.[5] Other constituents occasionally found in gall-stones are zinc (Thudichum and Maly), iron, copper, and manganese (Bley,[6] Wurzer[7]), fats (v. Planta and Kekule), silica (Pleischl,[8] Bley), uric acid (Stockhardt,[9] Marchand[10]), and in cases of typhoid fever and tuberculosis fat-globules (Gorup-Besanez[11]).

The Secretion of the Gall-bladder

B. Birch and H. Spong[12] obtained this secretion in two cases of biliary fistula in human beings, in which the bile channels were completely shut off from the gall-bladder. The amount secreted daily was 20 to 30 c.c. The fluid had the same characters in both cases; it was clear or faintly opalescent, viscid, and had a specific gravity of 1011 or 1012. It was always distinctly alkaline. It contained 2 per cent. of solids; 1·2 per cent. organic (mucin and a trace of proteid); 0·8 per cent. inorganic, of which the most abundant salt was sodium chloride. They do not regard this fluid as playing any important part in the digestive process.

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[1] Austin Flint, jun. *Recherches exp. sur une nouvelle fonction du foie*, Paris, 1868.

[2] *Ann. Chem. Pharm.* lxxxvii. 367.

[3] *Quart. Journ. of the Chem. Soc.* 1864.

[4] *Ann. Chem. Pharm.* clxxv. 76.

[5] Lehmann-Gmelin's *Lehrbuch*, viii. 45.

[6] *Journ. prakt. Chem.* i. 115.

[7] Schweigg, *Journal*, viii. 65.

[8] *Kastner's Archiv*, viii. 300.

[9] *Diss.* Lipsiæ, 1832.

[10] *Zeit. rat. Med.* iv. 114.

[11] *Lehrbuch*, p. 585.

[12] *Journ. of Physiol.* viii. 378.

The Invertebrate Liver

The so-called liver of invertebrate animals appears, in those cases in which an examination of its properties has been made, to fulfil the functions of a pancreas. A. B. Griffiths[1] found that the secretion of the 'liver' of the limpet, like that of cephalopods, converts starch into sugar, forms an emulsion with fats, and a soluble ferment extracted from the glands converts fibrin into peptone, leucine, and tyrosine. The secretion itself contains proteids, leucine, and tyrosine, but no biliary acids. Glycogen also could not be detected in either the organ or its secretion. The glycogenic function of the vertebrate liver is performed in molluscs by the connective-tissue-cells (Blundstone[2]).

Whether the gland that secretes the ink in sepia corresponds to a liver is a matter of doubt. The secretion is not digestive; it is used to colour the sea-water and cover the flight of the animal. It has been investigated by Schwartzenbach[3] and Hosacus,[4] who find the black pigment is its chief constituent (80 per cent. of the dry solids): there are also small quantities of a mucinoid substance, carbonate of calcium and magnesia, sulphate and chloride of sodium. Nencki and Sieber[5] have recently separated from the pigment an acid sepiaic acid, containing carbon, hydrogen, oxygen, nitrogen, and sulphur.

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[1] *Proc. Roy. Soc.* xlii. 392; *Proc. Roy. Soc. Edin.* xiii. 120.

[2] *Proc. Roy. Soc.* xxxviii. 442.

[3] *Liebig's Jahresb.* 1862, p. 539.

[4] *Arch. d. Pharm.* (2), cxx. 27.

[5] Nencki and Sieber. *Chem. Centralbl.* 1888, p. 587.

CHAPTER XXXV

PUTREFACTIVE PROCESSES IN THE INTESTINE

THE ancients regarded the whole digestive process as one of the nature of putrefaction; they used the term no doubt in a loose sense, but the earliest experiments of Réaumur, Spallanzani, and Beaumont showed that in the stomach at least there is no formation of malodorous gases, the presence of which is the most palpable evidence of putrefaction. This has since then been a matter of common observation; in certain disordered conditions of the stomach, gas-forming fungi may flourish and cause flatulence and eructations (p. 650), but during the normal digestive process in the stomach these are absent. Since we have known more about putrefaction and its causes, it has been found that bacteria do not flourish readily in acid media; *a priori* then we should not expect them to be active in the stomach. The actual investigation of the question has been made by Harris and Tooth,[1] who, using the latest bacteriological methods, have been able to demonstrate satisfactorily that the general belief in the absence of the activity of micro-organisms during gastric digestion is well founded. Straus and Wurtz[2] have found that gastric juice is an actual germicide, and destroys the bacillus anthracis, the cholera bacillus, and many others.

In the intestine, however, especially in the large intestine, putrefactive processes always occur. The bacteria are introduced with the food, but escape the direct action of the gastric juice. They may be diminished by purging, which produces rapid removal of the products of putrefaction, or by the administration of antiseptics; the use of these, however, in man is limited; large doses of iodoform or calomel, such as Baumann[3] and Moran[4] administered to dogs with success, would be exceedingly dangerous to use in human beings.

We have already seen that these processes are kept within normal limits by the natural antiseptic, the bile. Within such limits putrefaction is probably a useful process, acting on food very much in the same way as does the pancreatic juice. In many cases the organisms exert a peptonising action, and only seldom a diastatic action (W. Miller[5]).

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[1] *Journ. Physiol.* ix. 220.

[2] *Archives de méd. expérimentale*, 1890; *see Brit. Med. Journ.* vol. i. 1890, p. 252. *See* also Falk, *Virchow's Archiv*, xciii. 117; Frank, *Deutsch. med. Wochensch.* 1884, No. 24.

[3] *Zeit. physiol. Chem.* x. 123.

[4] *Ibid.* p. 318.

[5] *Chem. Centralbl.* 1886, p. 580.

Vignal[1] separated an enormous number of microbes from the fæces, six of which are found in the mouth also, and many of them have considerable digestive action. Many are fat-splitting. Other organisms bring about the formation of leucine and tyrosine, indole and skatole, lactic and butyric acids, &c. A useful function fulfilled by the organisms appears to be the destruction of poisonous substances, such as choline, the alkaloid derived from lecithin. It is possible that if other alkaloids (leucomaines) are formed by the processes occurring in the intestines these also are destroyed, for they are absent in the normal excretions. It need hardly be said that an excessive amount of putrefactive change in the intestines is injurious, producing distension of the abdomen by the gases which accumulate, and other forms of discomfort. The amount of putrefactive change occurring in the alimentary canal or elsewhere in the body as in putrid abscesses is best measured by the amount of certain products in the urine. These are termed ethereal sulphates; the indole, skatole, cresol, phenol, and a few other substances formed by putrefaction are absorbed in very large measure, and excreted in the urine as combined sulphates. The methods of estimating these will be described under Urine.

The gases of the intestinal canal have been analysed by Planer,[2] Ruge,[3] and Hofmann.[4] They vary a good deal with the diet. The following are Planer's numbers (from dogs) in 100 volumes of the mixture of gases:—

Gases	Small intestine			Large intestine	
	Meat diet	Bread diet	Vegetable diet	Meat diet	Vegetable diet
CO_2 . .	40·1	38·8	47·2	74·2	65·1
H_2 . .	13·9	6·3	48·7	1·4	2·9
H_2S . .	—	—	—	0·8	—
O_2 . .	0·5	0·7	—	—	—
N_2 . .	45·5	54·2	4·0	23·6	5·9

The following are Ruge's figures; the gases were obtained from human beings:—

Gases	Milk diet	Meat diet	Vegetable diet
CO_2	9 to 16	8 to 13	21 to 34
H_2	43 to 54	0·7 to 3	1·5 to 4
CH_4	0·9	26 to 37	44 to 55
N_2	36 to 38	45 to 64	10 to 19

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[1] *Compt. rend.* cv. 311.

[2] *Sitzungsber. Wien. Akad.* xlii.

[3] *Ibid.* xliv.

[4] *Wien. med. Wochensch.* 1872, No. 24.

Oxygen and sulphuretted hydrogen were found in traces only; Hofmann found no marsh gas in rabbits.

The carbonic acid, as is seen in the above tables, is always present in large quantities, especially in the large intestine, and especially when the diet is vegetable. Its sources are the decomposition of carbonates, acetates, and lactates in the food, the alcoholic fermentation of dextrose in the intestine, the putrefaction of carbohydrates (especially cellulose) and proteids, the butyric fermentation of lactic acid, and the putrefaction of choline. The enormous quantity of gas discharged in cases of hysterical flatulence consists largely of carbonic acid; it is possible it may have simply diffused from the blood-vessels.

The hydrogen is most abundant on a milk diet; its source is the butyric acid fermentation of lactic acid (p. 103).

The marsh gas is derived from the decomposition of acetates and lactates. Hoppe-Seyler[1] represents the decomposition of calcium acetate by the equation $(C_2H_3O_2)_2Ca + H_2O = CaCO_3 + CO_2 + 2CH_4$. It is also derived from the decomposition of cellulose (Hoppe-Seyler,[2] Tappeiner,[3] Henneberg and Stohmann[4]). Hoppe-Seyler's formula for the reaction is $C_6H_{10}O_5 + H_2O = 3CO_2 + 3CH_4$. Henneberg and Stohmann consider that hydrogen, acetic acid, and butyric acid are also formed, their equation for the reaction being $21C_6H_{10}O_5 + 11H_2O = 26CO_2 + 10CH_4 + 6H_2 + 19C_2H_4O_2 + 13C_4H_8O_2$; whichever equation is correct, the fact remains unaltered that a vegetable diet is that which yields most marsh gas. A third and small source of marsh gas is from the choline of lecithin (Hasebroek[5]).

The nitrogen is derived chiefly from the swallowed air; the oxygen is largely absorbed; nitrogen is also contained in the ammonia, which is the result both of pancreatic digestion and putrefaction of proteids.

The hydrogen sulphide is derived wholly from the putrefaction of proteids.

We may in conclusion briefly glance at the matter of putrefaction from another point of view, namely, its action on each class of the proximate principles of food.

Action on fats.—This is a fat-splitting action, exactly similar to that produced by the steapsin of the pancreatic juice. Putrefaction in addition produces lower acids (valerianic, butyric, &c.) of the fatty series. Lecithin is similarly decomposed into its acid (glycero-phosphoric) and choline which then breaks up into carbonic acid, marsh gas, and ammonia.

Action on carbohydrates.—The chief fermentation here is the lactic acid followed by the butyric acid fermentation (*see* p. 103).

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[1] *Zeit. physiol. Chem.* ii. 561.

[2] *Ibid.* x. 201, 401.

[3] *Zeit. Biol.* xx. 52; xxiv. 105.

[4] *Ibid.* xxi. 613.

[5] *Zeit. physiol. Chem.* xii. 148.

With regard to cellulose it may be here stated that putrefaction is the only known change that this constituent of food undergoes in the alimentary canal.[1] Henneberg and Stohmann nevertheless consider it a source of energy. An important practical point in cattle feeding, whether cellulose economises the decomposition of proteid, has not yet passed beyond the regions of dispute (v. Knierem,[2] Weiske, and others[3]).

Action on proteids.—The antipeptone is decomposed with more difficulty than the hemipeptone. The products of putrefaction of proteids are ammonia, sulphuretted hydrogen, ammonium sulphide, volatile and fatty acids; amines and amido-acids, especially leucine and tyrosine; indole, skatole, phenol, and cresol, phenyl-propionic, and phenyl-acetic acids, and the aromatic oxy-acids, hydroparacumaric and parahydroxyphenylacetic acids. The presence of these numerous acid compounds, especially of lactic acid, gives the contents of the large intestine, as a rule, an acid reaction. The presence of indole and skatole gives the fæces their characteristic odour; they are, however, very largely absorbed and excreted as ethereal sulphates in the urine.

With regard to the production of indole, Harris and Tooth[4] found that its appearance, and that of its allies, is capricious, and can be easily prevented in artificial pancreatic digestion. The smallest amount of mercuric chloride or phenol, even if not sufficient to render the fluid aseptic, prevents the formation of these substances. Whenever indole is present, however, large numbers of all sorts of bacteria are present also; still it may be absent even if swarms of micro-organisms are present. It thus appears that there are special indole-forming organisms. As a result of inoculation experiments, it was found that indole was formed from peptone, not from leucine and tyrosine.

It is interesting to note that certain products of putrefaction, especially phenol or carbolic acid, and cresol are antiseptics; the microbes thus produce compounds which, if allowed to accumulate, would ultimately destroy their life.

It is considered by certain observers that the production of poisonous alkaloids is a normal process in the alimentary canal, that these are absorbed, and if excessive in amount may produce self-poisoning or 'auto-intoxication.' As a rule they are excreted, however, by the kidney, and thus the body generally escapes their poisonous action (Bouchard). Such a doctrine must be considered unproven for the present. The most careful of the numerous researches in this direction entirely negative the idea. Ptomaines are absent, not only in normal urine and fæces, but also in these excretions in various diseases. There is, however, some evidence of their formation in typhoid fever, cholera, and cystinuria. We have seen that under normal circumstances choline, a typical instance of a poisonous animal alkaloid, is broken up into simple non-poisonous products by the intestinal bacteria; and it is exceedingly probable that if other alkaloids are produced by bacteria in the intestine, they also are promptly destroyed by other species of the same micro-organisms (*see* also Chapter XIII).

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[1] Bunge surmises (*Physiol. Chem.* 192) that the epithelium cells of the intestine may have a similar action on cellulose. He also dwells (p. 81) on the important action of cellulose as a mechanical stimulus to peristalsis.

[2] *Zeit. Biol.* xxiv. 293. [3] *Ibid.* xxii. 373. [4] *Journ. of Physiol.* ix. 230.

CHAPTER XXXVI

THE FÆCES

THE fæces consist of the indigestible and undigested portions of the food, products formed from food-stuffs by the digestive ferments (indole, skatole, soaps, &c.), and certain constituents of the digestive secretions (mucin, altered bile-pigment, &c.).

The amount of the fæces varies with the amount and character of the food. Over-eating entails voluminous excrements, since, though much of the food taken may be digestible, it escapes digestion and absorption simply because its amount is too great for the digestive ferments to act upon, or for the absorbing surface to come in contact with. On a mixed diet of moderate amount in man, Liebig calculated that the weight of the fæces is one-seventh to one-eighth of the food taken. Calculating both food and fæces in the dry state, Bischoff and Voit found in dogs that with a nitrogenous diet the fæces weighed one-tenth to one-fourteenth, with a bread diet one-sixth to one-eighth of the food. The amount of water in the fæces varies considerably in health from 68 to 82 per cent. In diarrhœa it is more abundant still.

The constituents of the fæces may be classified as follows:—

1. Undigested foods: fats, carbohydrates, and proteids, if any of these are present in excess in the food. On a moderate diet, unaltered proteid is never found.

2. Indigestible constituents of the food: cellulose, keratin, mucin,[1] chlorophyll, gums, resins, cholesterin.

3. Constituents digestible with difficulty: uncooked starch, tendons, elastin, nuclein, various phosphates, and other salts of the alkaline earths.

4. Products of decomposition of the food: indole, skatole, phenol, &c.; fatty acids, formic, acetic, butyric, isobutyric,[2] caproic, valerianic; other acids, lactic, malic, succinic, &c. Some of these acids are free; some in combination with ammonia and other bases; hæmatin from hæmoglobin;[3] soaps, especially calcium and magnesium soaps of oleic, palmitic, and stearic acids (the soluble soaps are, of course, to a large extent

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[1] Mucin which has been separated out by means of lime water and acetic acid is readily digestible by artificial pancreatic juice (*see* p. 481). Mucin as contained in mucus, however, appears to be quite unaltered by the natural juices.

[2] Brieger, *Ber. deutsch. chem. Ges.* x. 1027.

[3] Hoppe-Seyler, *Physiol. Chem.* p. 339.

absorbed); stercorin, a product of decomposition of cholesterin: this substance was described by Flint,[1] but its existence is very doubtful: excretin ($C_{20}H_{36}O$), another doubtful substance described by Marcet.[2]

5. Bacteria of all sorts and *débris* from the intestinal wall: cells, nuclei, mucus, &c. L. Hermann[3] found in a loop of intestine separated in the manner of Thiry and Vella that at the end of some weeks it was filled with bacteria, cellular *débris*, and often fat, the whole mass having a fæcal appearance.

6. Bile residues: these are mucin, traces of bile-acids and their products of decomposition; cholesterin and lecithin, the latter in traces only are also found; these two substances also partly owe their origin to the ingested foods. The bile-pigments as such are not present, but are changed into a substance like hydro-bilirubin, which is called *stercobilin.*[4] Stercobilin may originate also from the hæmatin in the food (MacMunn[5]). Hoppe-Seyler,[6] however, who made experiments on dogs, found that hæmatin is easily discoverable in the fæces, and regards it as improbable that stercobilin originates from the hæmoglobin of the food. This subject merits renewed study, and the experiments should be made on animals in which no bile is allowed to enter the intestine. The meat of the food cannot, however, be a large contributor to the pigments of the fæces, as the stools of jaundiced persons are clay-coloured even if they are on a meat diet. Hydro-bilirubin and stercobilin are usually considered to be produced by reduction processes: MacMunn, however, regards the formation of stercobilin as one of intermediate oxidation; by further oxidation it may be transformed into a substance like choletelin, the most highly oxygenised product of the bile-pigment with which we are acquainted. T. J. Walker[7] has recorded two cases in which the liver was apparently healthy, but the pancreatic duct was occluded; the fæces in these cases were free from stercobilin, being clay-coloured as in jaundiced persons. He therefore concludes that the pancreatic ferment is in some way necessary for the formation of the fæcal pigment.

Stercobilin may be most readily prepared by extracting the fæces with acidulated alcohol (17 parts of rectified spirit to 3 of sulphuric acid); the extract is diluted with water, and shaken with chloroform: the chloroform dissolves out the pigment and may be driven off by evaporation.

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[1] *Recherches exp. sur une nouvelle fonction du foie*, Paris, 1868.

[2] *Ann. de chem. et de phys.* lix. 91.

[3] *Du Bois Reymond's Archiv*, 18[illegible].

[4] Vaulair and Masius, *Centr. med. Wiss.* 1871, No. 24.

[5] *Journ. of Physiol.* x. 115.

[6] *Physiol. Chem.* p. 339.

[7] *Medico-Chirurgical Trans.* vol. lxxii. 1889, p. 257.

Before proceeding to describe the spectroscopic appearances of this substance it must be acknowledged that as yet spectroscopic analysis is the only method yet applied to this and related pigments (hydro-bilirubin, urobilin, &c.); it is possible in the future that other methods of investigation may confirm or correct the knowledge obtained by the spectroscope. Another possible source of error is the admixture of unchanged hæmatin with such pigments, and a third difficulty arises from the fact that there are probably intermediate products between bilirubin and stercobilin which occur in different proportions in different preparations. This last assumption is confirmed by the differences obtained in measurements of the bands of stercobilin in different preparations. One of these intermediate products appears to be absorbed, carried to the liver, and there excreted into the bile as biliary urobilin (p. 685); by further oxidation biliary urobilin can be artificially changed into a pigment closely resembling stercobilin.

The absorption spectrum of stercobilin is practically identical with that of hydro-bilirubin (fig. 88, spectrum 3, p. 685). We have seen, however, that hydro-bilirubin after treatment with zinc chloride and ammonia shows a green fluorescence and a three-banded spectrum; stercobilin, on the contrary, though it shows the same fluorescence, gives a four-banded spectrum. There are also certain differences in the spectra of the two substances after treatment with other reagents, such as soda, or zinc chloride by itself, or ammonia by itself. The spectroscope thus teaches us that the two substances cannot be identical. Still more does the spectroscope teach us the non-identity of either of these pigments with urobilin. Jaffé[1] and Maly[2] first described urobilin, and considered that it originated from bilirubin, that bilirubin was changed into hydro-bilirubin in the intestine, and then partly absorbed and excreted in the urine. Subsequent investigations have, however, shown that there are two pigments or their chromogens in the urine which have each received the name urobilin; one is normal urobilin, which shows the same spectrum as choletelin, that is one band only (at F); the other pathological urobilin which occurs in certain diseased conditions is possibly identical with stercobilin, and no doubt originates in the intestine as Maly considered.

Normal urobilin does not necessarily arise in the intestine from stercobilin; in Copeman and Winston's case of biliary fistula,[3] no bile entered the intestine, but the urine was not colourless; it contained ordinary urobilin. In cases of extravasation of blood, the destruction of blood-pigment may give rise to pathological urobilin in the urine,[4] and moreover normal urobilin was obtained artificially by MacMunn by acting on acid hæmatin with hydrogen peroxide. Pathological urobilin is regarded by MacMunn as a less highly oxidised product than normal urobilin. It thus appears that if the urine pigment be formed in the liver, it is unnecessary for it to go through the stage of bile-pigment, though this stage probably occurs under normal circumstances. This subject will be more fully dealt with under Urine (Chapter XLI).

Meconium

The meconium, or the contents of the intestine of new-born children, is a greenish-brown, almost black, viscid material. Its reaction is generally acid. On microscopic examination it shows leucocytes, often stained green, columnar epithelium cells from the intestinal wall, fat-globules, and crystals of cholesterin. Zweifel found it contained 20 to 27 per cent. of solids, of which 1 per cent. was inorganic, the remainder organic ; the percentage of fat and fatty acids was 0·75 ; that of cholesterin was also 0·75. The chief organic constituents are the bile-salts, more or less changed ; the bile-pigments bilirubin and biliverdin, not changed at all, and mucin.

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[1] *Centralbl. med. Wiss.* 1868, p. 241.

[2] *Ann. Chem. Pharm.* clxi. 368; clxiii. 77.

[3] *Journ. Physiol.* x. 213.

[4] Cases recorded by MacMunn, *Ibid.* p. 83.

The inorganic constituents are phosphates and sulphates of magnesium and calcium, oxide of iron and sodium chloride.

The most remarkable difference between meconium and fæces is in the pigment. In meconium, stercobilin is absent ; in addition to biliverdin and bilirubin, it contains a small quantity of a purplish pigment, which gives a narrow absorption band before D, and another darker and wider between D and E, which is probably an oxidation product of bilirubin (Hoppe-Seyler).[1]

In fact, meconium is, as Mott[2] puts it, little else but concentrated bile.

Pathological Alterations in the Fæces

Section of the nerves going to a loop of intestine paralyses the blood-vessels, and causes an abundant watery exudation. If these nerves contain fibres which are secretory in function, this increased flow of fluid may be in part a paralytic secretion of the intestinal glands (Moreau[3]).

Purgatives act in various ways, some exciting a flow of fluid into the intestine, some increasing peristalsis, others acting in both ways. The excito-secretory action of saline purges, like magnesium sulphate, is probably due to their irritant properties, and not simply to osmosis. the low diffusibility of the salt, however, impedes the absorption of the secreted fluid (Hay[4]).

The diarrhœa of certain diseases (typhoid, cholera, dysentery, &c.) is probably due to specific poisons produced by bacteria. Ordinary diarrhœa is due to the irritant action of bad or indigestible food, or to accumulation of hard fæces, or it may be produced in certain forms of emotion.

The rice-water stools of cholera contain a low percentage of solids, very little proteid, little or no blood, a vast amount of intestinal epithelium, leucine, and tyrosine, and perhaps certain ptomaines.

Blood and pus appear in dysentery, and occasionally in typhoid fever. If the blood is small in amount, the hæmatin which is formed gives the stools a dark, almost black colour. If the amount of blood is large, as in ulceration into a blood-vessel, the corpuscles and hæmoglobin are for the most part unchanged.

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[1] *Physiol. Chem.* p. 340.

[2] *Practitioner*, Aug. 1890.

[3] *Compt. rend.* 1858, p. 554.

[4] *Brunton's Materia Medica*, p. 342.

Typhoid stools contain abundance of ammonium carbonate, and ammonio-magnesium phosphate often in crystals. Skatole is absent (Brieger[1]).

In intestinal catarrh the fæces are watery, and contain albumin, an increased percentage of salts, urea, and alloxan. The urea is also increased in uræmia; it may, however, be converted into ammonium carbonate. The chief salts in the stools in all cases of diarrhœa, cholera included, are chlorides of sodium and potassium; the amount of chlorides in the urine is correspondingly diminished (Schmidt[2]).

In jaundice, bile is absent, and the stools are hard and clay-coloured. T. J. Walker recorded two cases in which clay-coloured stools occurred, though the liver was apparently healthy; the pancreatic duct was, however, occluded. In some forms of diarrhœa there is an excessive amount of bile present.

In cases where either the bile or pancreatic secretion is diminished or absent, the fat of the food is in great measure not digested, and passes away in the fæces. In some of these cases the fats or their soaps are found in a crystalline condition in the fæces (Oesterlein,[3] Stadelmann[4]).

The administration of the salts of mercury or iron causes the fæces to be black from the formation of the sulphides of those metals.

Gall-stones may be present, having passed from the gall passages into the intestines.

Scybalæ are hard masses of fæces containing a good deal of dried mucus.

Intestinal concretions consist generally of earthy phosphates; but they may contain chiefly organic matters, fat, hair, vegetable fibres, and in some animals (antelopes) two special components, named *lithofellic* ($C_{20}H_{36}O_4$) and *ellagic* ($C_{14}H_6O_8 + 2H_2O$) acids, have been described (Ettling and Will, Gorup-Besanez[5]).

Such very briefly is an enumeration of the pathological conditions met with in the fæces. To the practical physician, the subject of properly recognising these is a matter of paramount importance, and for further imformation on the subject, the reader is referred to works on materia medica, medicine, and pathology.

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[1] *Ber. d. chem. Ges.* 1877, p. 1031.

[2] *Characteristik der Cholera*, Leipzig, 1850.

[3] *Mitth. a. d. med. Klinik in Würzburg*, i. 1.

[4] *Archiv f. klin. Med.* xl. 372.

[5] *Lehrbuch*, 1874, p. 557.

CHAPTER XXXVII

ABSORPTION

Food is digested in order that it may be absorbed. Certain changes are produced by the action of the digestive secretions on the food, by means of which it is reduced to such a condition that it may pass more easily into the blood-vessels and lacteals of the intestinal walls. In the mouth and œsophagus, the thickness of the epithelium and the quick passage of the food through these parts reduce absorption to a minimum. Absorption takes place rapidly from the stomach ; it is stated that most of the peptone formed in the stomach is absorbed before the chyme passes into the duodenum. The small intestine with its folds, and villi to increase its surface, is, however, the great place for absorption ; and although the villi are absent from the large intestine, absorption occurs there also, but to a less extent.

Some foods, such as water and certain salts (sodium chloride, &c.), are not acted on by digestive juices, but are absorbed unchanged. The organic foods, however, undergo a change from a colloid to a diffusible condition ; thus proteids are changed into peptone, and starch into sugar : these soluble substances diffuse into the neighbouring vessels. The fats undergo a double change ; the smaller amount is saponified, and soluble soaps are absorbed like other soluble materials ; the greater part of the fat is, however, emulsified, that is, reduced to a fine state of subdivision ; the minute fat-globules pass into the vessels by a mechanism which will require special description.

The question as to whether the lymphatics are the only absorbents was settled by Magendie, who showed that if the thoracic duct of an animal be ligatured, and a soluble poison introduced into the intestine, the animal dies quickly because the poison has been taken into the blood-vessels.

Absorption is partly a physical process, namely, that of diffusion. Water, salts, and sugar pass out of the intestinal canal into blood or lymph, when the fluid in the intestine is richer in those substances than the blood or lymph ; and the greater the difference between the contents of the intestine and that of the vessels, the more rapidly does diffusion occur. The process is thus not simply one of filtration under pressure caused by the movements of the intestine. The rate of diffusion is increased by the fact that all the fluids concerned are in motion, and so new layers of fluid are successively being brought into juxtaposition.

Absorption is by no means a mere physical process: we must also take into account the fact that the cells through which the fluids pass are living, and have a power of, not only selecting materials for absorption, but also of changing those substances while in contact with them. It is in the absorption of proteids and fats particularly that the vital properties of the cells come into play.[1] The cells are of two kinds: (1) the columnar epithelium that covers the surface, (2) the lymph-cells in the lymphoid tissue of the corium. There are also special collections of lymphoid tissue called the solitary and agminated glands. Stöhr[2] has recently pointed out that the lymph-cells make their way out between the epithelial cells into the intestine, especially during digestion; and perhaps these may take a greater part in absorption processes than has been hitherto considered to be the case.

Absorption of carbohydrates.--The sugar formed by the salivary and pancreatic juices from starch and glycogen is maltose: that found in the blood, and in the lymph, is glucose. The inversion of maltose into glucose appears to be brought about by the succus entericus, or it may partly occur in the passage of the sugar through the epithelial cells of the intestine.

Lactose is changed for the most part into glucose in a similar way before absorption.[3] Cane sugar is inverted into glucose before absorption, but here again small quantities may be absorbed as such, as it has been found in the portal blood,[4] and in the urine.[5] Small quantities of dextrin have been found in portal blood. Komanos found small quantities of inulin in the portal blood after ingestion of that substance.[6]

It may thus be stated in general terms that *carbohydrates are absorbed as glucose, which passes chiefly into the blood-stream.*[7] It only penetrates the villi to the central lacteals when present in excess (*see* p. 337).

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[1] It has been already pointed out (pp. 14, 15) that osmosis through living membranes is modified by what we must call for want of a better name, the vitality of its cells. The selective power of the cells of the alimentary tract was well shown by Tappeiner (*Wien. Sitzungsber.* lxxvii. p. 281; 1878), who found that bile salts were absorbed by the ileum, but not by the duodenum or jejunum. Susini (*Journ. de l'Anat.* 1868, p. 144) also found that potassium ferrocyanide passes through the intestinal wall with greater ease than through the stomach wall. *See* also Reid (*Journ. of Physiol.* xi. 312).

[2] *Archiv für mikros. Anat.* xxxiii.

[3] The cases in which it has been found in the urine are nursing mothers (Hofmeister, *Zeit. physiol. Chem.* i. 101). In such cases the sugar would not come from the alimentary canal.

[4] Drosdoff, *Ibid.* Heft iv.

[5] Seegen, *Pflüger's Archiv*, xl. 48.

[6] *Diss.* Strasburg, 1875.

[7] Pavy's statement (*Internat. Med. Congress*, 1890) that the sugar in the portal vein is maltose, must, for the present, be accepted with caution.

Absorption of proteids.—There is evidence to show that a certain amount of proteid is absorbed unchanged. Dr. D'Arcy Power, for instance, after swallowing a dozen raw eggs, found egg-albumin in his urine.[1]

Feeding patients *per rectum*, when apparently proteolytic ferments are absent, has been followed by the absorption of the albuminous food thus injected. Czerny and Latschenberger's[2] experiments on a man with an artificial anus in the sigmoid flexure are not open to the objection that the pancreatic ferment extends into the rectum. In this case the rectum was well syringed out and then filled with solution of proteid. After twenty-four hours it was found that 60 to 70 per cent of this had disappeared. Voit and Bauer[3] and Eichhorst[4] have made confirmatory experiments in dogs.

We shall see that the absorbent elements of the intestine have the power to take up unaltered fat; there is, therefore, nothing wonderful in the fact that they can take up unaltered proteid.

There is, however, little doubt that most proteid is absorbed as peptone, or as peptone and albumose. We are, however, confronted with the remarkable fact that no albumose or peptone is discoverable in the blood or lymph, even during the periods of most active digestion. Maly,[5] Plósz and Gyergyai,[6] and Adamkiewicz[7] were all agreed on this point, though they differed as to where the change into the blood-proteids occurred. Some of these earlier investigators thought the liver effected the change, but this view is negatived, as portal blood is as free from albumoses and peptones as the hepatic blood. Schmidt-Mulheim[8] and Fano[9] were inclined to think that the change occurred in the blood itself, since a solution of commercial peptone injected into the blood-stream disappeared from the blood in a few minutes.

This power of blood to destroy peptone is a property of the blood within the vessels; it is lost when the blood is shed. What actually becomes of peptone thus injected is a mystery; Hofmeister[10] found that from two-thirds to two-fifths of the peptone injected appeared in the urine; thus these substances must exist in the blood either as such, or very loosely combined. In some animals in which so much peptone was injected as to cause a fall of blood-pressure sufficient to stop the secretion of urine, the peptone disappeared from the blood all the same. Hofmeister himself considers that the peptone collects in certain organs, such as the kidneys, for when they resume work they secrete small quantities of peptone; he also supposes that in the blood itself the white blood-corpuscles have the power of combining with the peptone.

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[1] *Lauder Brunton's Disorders of Digestion*, p. 37.

[2] *Virchow's Arch.* lix. 161.

[3] *Zeit. Biol.* v. 562.

[4] *Pflüger's Archiv*, ix. [illegible]

[5] *Pflüger's Archiv*, 1874, p. 385.

[6] *Ibid.* p. 325.

[7] *Die Natur und der Nahrwerth des Peptons*, 1877.

[8] *Du Bois Reymond's Archiv f. Physiol.* 1880, p. 33.

[9] *Ibid.* 1881, p. 277.

[10] *Zeit. physiol. Chem.* 1881, p. 27.

Later Hofmeister[1] found in the blood of animals during digestion small quantities of peptone; but these were sometimes absent. Schmidt-Mulheim, Plósz and Gyergyai[2] and Drosdoff[3] had before this found traces of peptone in portal blood. This was, however, in the days before the use of ammonium sulphate as a reagent for the separation of peptones. Neumeister,[4] who has employed this reagent, proves most conclusively that both peptones and albumoses are always absent from both blood and lymph, even during the most active periods of digestion. It is as well for us that they are, as they are most violent poisons, causing a rise of temperature, a fall of blood-pressure, and a change in the blood, rendering it uncoagulable.

Neumeister also found that, although it is possible after injection to recognise the presence of albumoses and peptones by their effects in rendering the blood uncoagulable, they cannot be demonstrated there by chemical means; their secretion by the kidneys begins ten minutes after injection. In the dog he found that the albumoses underwent hydration before appearing in the urine, the primary albumoses appearing as deutero-albumose, the deutero-albumose as peptone. Probably this digestion occurs by means of the pepsin secreted by the kidneys in the urinary tubules, where there is momentarily a formation of free acid. In the rabbit no such change occurs; the urine contains no pepsin in this animal, and the albumoses injected into the circulation are secreted as such.

Where, then, during normal digestion does the change from peptone into the blood proteids occur? It must occur during the actual process of absorption; though whether the epithelium-cells, or the lymph-cells, or both are the active agents in producing the dehydration there is at present no evidence to show.

There is, however, evidence to show that the mucous membrane as a whole has this power. Hofmeister[5] found that the mucous membrane of the stomach and intestine are the only parts of the body in which a supply of peptone is always found during digestion. A stomach recently removed from an animal has also the power of reconverting peptone into native proteid. V. Ott[6] and others, who have carried out researches in Kronecker's laboratory, use the word *serum-albumin* synonymously with the proteids of the blood-plasma. The actual proof of the obtaining of serum-albumin is in their case by no means satisfactory; it is not a chemical, but a physiological one. A solution of 'serum-albumin' artificially circulated through a frog's heart has the power of keeping it beating; this power is not possessed by a similar solution of peptone. If, however, the solution of peptone be placed into the stomach of a living dog, and withdrawn in a few minutes, it is again capable of keeping the heart beating. This is regarded as sufficient proof that the stomach had in this time reconverted or regenerated the 'peptone' into 'serum-albumin.' Miss Popoff[7] showed that the same result followed if, instead of putting the solution of peptone into the stomach, it were allowed to remain in a loop of intestine separated from the rest of the alimentary tract by a Vella's fistula; or even if it were placed in contact with pieces of mucous membrane removed from a recently killed animal. Peptone produced by the pancreatic ferment, however, was not regenerated in this way. These experiments are not altogether satisfactory, as they entirely leave out of account Ringer's important results, showing the great effect produced by minute doses of salts on the frog's heart (*see* p. 256).

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[1] *Zeit. physiol. Chem.* 1882, p. 51.

[2] *Pflüger's Archiv*, x. 536.

[3] *Zeit. physiol. Chem.* 1877-8, p. 216.

[4] *Zeit. Biol.* xxiv. 272.

[5] *Zeit. Physiol. Chem.* iv. v. vi.; *Arch. f. exp. Path. u. Pharm.* xix. *See* also Salvioli, *Du Bois Reymond's Archiv*, Suppl. 1880, p. 112.

[6] *Archiv f. Physiol.* 1883, p. 89.

[7] *Zeit. Biol.* xxv. 427; *see* also Miss Brinck, *Ibid.* 453.

All these facts taken together constitute a very strong chain of evidence that under normal circumstances peptone is 'regenerated,' not while it still remains in the cavity of the stomach or the intestine, and not after it reaches the blood or lymph, and still less the liver, but during its passage through the cells of the mucous membrane.

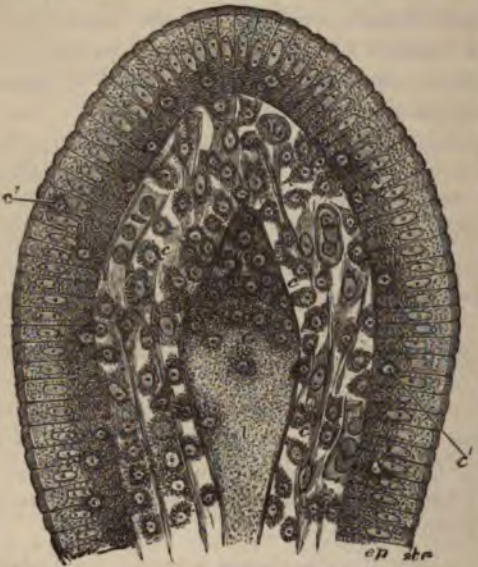

FIG. 89.—Section of the Villus of a Rat killed during fat-absorption (E. A. Schäfer): *ep*, epithelium; *str*, striated border; *c*, lymph-cells; *c'*, lymph-cells in the epithelium; *l*, central lacteal containing disintegrating lymph-cells.

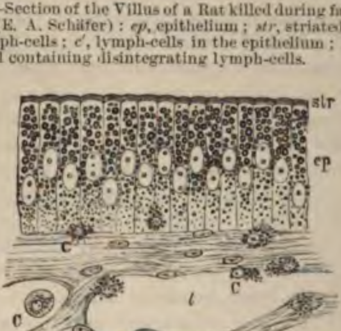

FIG. 90.—Mucous Membrane of Frog's Intestine during fat-absorption (E. A. Schäfer): *ep*, epithelium; *str*, striated border; *c*, lymph-cells; *l*, lacteal.

The absorption of fat.—The way in which minute fat-globules pass from the intestine into the lacteals has been the subject of much controversy. Instead of entering into this controversy I propose here to give a résumé of Prof. Schäfer's recent paper on the subject.[1]

For the purpose of studying the course which fatty particles take, an animal is killed three or four hours[2] after a meal of fat, previous to which the animal had been fasting. Small pieces of the mucous membrane are snipped off, and placed in 1 per cent. osmic acid solution, or examined fresh after teasing in serum. The portions in osmic acid are allowed to remain there forty-eight hours, and then broken up in water, or some may be imbedded and sections prepared. The fat-globules will be found both in epithelium-cells and lymph-corpuscles; the latter convey them to, and discharge them into, the central lacteal.

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[1] *Internat. Monatsschrift für Anat. u. Physiol.* ii. 6.

[2] In performing experiments on frogs, twenty-four hours at least should elapse before the animal is killed.

The columnar epithelium during absorption.—Each cell is composed of protoplasm, so soft and yielding, that the leucocytes that occur amongst the columnar cells are able to indent it. The attached end is *always* truncated and flattened where it rests against the basement membrane; it is *never* prolonged into an arborescent process which passes into the corium, and anastomoses with processes of lymph-cells, as some have supposed. The bright border is by no means firm, resisting or protective to the rest of the cell, but it appears to surpass the rest of the protoplasm in the readiness with which it imbibes fluid, and becomes swollen in consequence of such imbibition. Whatever may be true for the hypoblast of simpler animals (sponges, cœlenterates, &c.), in vertebrates, the border of the cell is never thrown into amœboid processes.

During fat absorption, these columnar epithelium cells become filled with fatty globules of variable size, but generally larger near their free borders (figs. 88 and 90). These globules pass down to the attached portion of the cell, the larger ones breaking down into smaller ones during this journey. How the fat actually gets into the cells is still unsolved.[1] Kühne considers that each globule of fat may be encased in an albuminous envelope which facilitates its adhesion to the epithelium cell. But, as in milk, the existence of such envelopes must still be a moot point. It is certainly necessary that the fat should have a melting point below that of the body; fats with higher melting points are not absorbed.[2]

Some have supposed that the cells have a further action in recomposing into fat those small quantities of fat which in the intestine have been broken up into fatty acids and glycerin.[3] Occasionally in frogs very little fat is absorbed, and none passes into the columnar cells; what is absorbed is taken up by the lymph-corpuscles between the epithelium cells.

The lymphoid tissue of the mucous membrane.—The presence of vast numbers of amœboid cells, not only in the lymphoid nodules of Peyer's patches and the solitary glands, but also in the general corium of the mucous membrane, becomes intelligible, when it is considered that they are actively concerned in promoting the absorption of the products of digestion. These cells are more numerous during absorption than at other times, and seem also to approach nearer the surface, making their way up between the epithelium cells. During absorption they are filled with fat-globules, which they take either from the intestine itself or from the epithelium cells; they then by amœboid movements work their way inwards, ultimately penetrating the wall of the lacteal. Arrived inside the lacteal, they disintegrate and discharge their cargo into the lymph-stream. The globules are by this time divided into immeasurably small ones, the molecular basis of chyle. In a section through a villus, as in fig. 89, they may be seen in all these different positions; in the lacteals they may be seen in various stages of disintegration.

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[1] Gruenhagen and Krohn (*Bied. Centr.* xviii. 617) found that fat-globules were taken up by the epithelium cells freshly detached by teazing from the frog's intestine. The cells never took up, however, the finest solid particles placed in contact with them.

[2] Arnschink, *Zeit. Biol.* xxvi. 534.

[3] Munk (*Virchow's Archiv*, xcv. 409), however, believes that this synthesis occurs in the lymph-corpuscles.

After an abundant fatty meal, the blood plasma is quite milky. This, however, is harmless, as the fat droplets are so small that they circulate without hindrance through the capillaries. Sometimes the fat enters the blood-stream under abnormal circumstances; for instance, from the marrow of a fractured bone; here the fat cells may block the capillaries in important areas, and cause serious symptoms, and even death. The fat in the blood after a meal travels through the walls of the capillaries, and is eventually stored up in connective-tissue cells (Bunge).[1]

Since fat is the only alimentary substance that can be detected after its absorption from the contents of the intestine, attention has been chiefly directed towards the investigation of the path taken by the absorbed fat-particles, but it is highly probable that the amœboid cells are actively concerned in promoting the absorption of alimentary substances of all kinds. The probable affinity between peptone and leucocytes has been already alluded to. Many observations have been accumulating recently, which show the importance of the part played by leucocytes in the promotion of various absorption processes. Of these, the most remarkable are those of Metschnikoff,[2] who describes the absorption of the tail of the batrachian larva as being the eating up of its several component tissues by numerous leucocytes, which take in fragments of muscle, nerve, and the rest, and transfer them to the body of the metamorphosing larva, submitting them to a sort of intracellular digestion on the way. The white blood-corpuscles seize and ingest foreign substances, or dead tissues such as vermilion particles, milk-globules, and even bacterial organisms, which are thus possibly rendered innocuous.[3]

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[1] *Physiol. Chem.* p. 221.

[2] *Biol. Centralbl.* iii. [illegible]

[3] Metschnikoff, *Virchow's Archiv*, xci. 3. *See* also Metschnikoff on 'Digestion in Sponges,' *Zeit. wiss. Zool.* xxxii. 371; Lendenfeld on 'Cœlenterates,' *Ibid.* xxxviii. [illegible]

PART V

EXCRETION

CHAPTER XXXVIII

THE URINE

THE KIDNEY

THE kidney is a compound tubular gland ; and the tubules of which it is composed differ much in the character of the epithelium that lines them in various parts of their course. The true secreting part of the kidney is the glandular epithelium which lines the convoluted portions of the tubules ; there is, in addition to this, what is usually termed a filtering apparatus ; tufts of capillary blood-vessels, called the Malpighian glomeruli, are supplied by afferent vessels from the renal artery ; the efferent vessels that leave these have a smaller calibre, and thus there is a high pressure in the vessels of the glomeruli themselves ; certain constituents of the blood, especially water and salts, pass through the thin walls of these vessels into the surrounding Bowman's capsule, which forms the commencement of each renal tubule. Bowman's capsule is lined by a flattened epithelium, which is reflected over the capillary tuft, and this epithelium prevents, to a great extent, the albuminous constituents of the blood from escaping. After ligature of the renal arteries, and subsequent restoration of the renal circulation, this epithelium becomes injured, and the urine is then found to be albuminous. During the passage of the water which leaves the blood at the glomerulus, throughout the rest of the renal tubule, it is in part reabsorbed, and in health it is also generally held that, if any albumin has filtered or diffused through, it also is absorbed, for normal urine is quite free from proteids. The urine, however, in its course through the tubules, not only loses, it also gains constituents, namely, various organic substances (urea, uric acid, &c.), and a certain proportion of salts and water, which are poured into it by the secreting cells of the convoluted tubules.

Bowman was the first to point out the double source of the constituents of the urine, and Ludwig, who first advanced the theory of reabsorption. Hüfner, from a comparative study of kidneys throughout the vertebrate kingdom, confirms Ludwig's views by pointing out that the tubules are long or short according as it is necessary or not for the animal that the water should be reabsorbed ; and Ribbert[1] has been able to collect the urine which had passed through the Malpighian glomeruli only, and to show that it is more watery than that secreted by the whole kidney.

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[1] *Virchow's Archiv*, 1883, p. 1

Heidenhain's experiments first demonstrated conclusively that the renal secreting epithelium takes part in the process of urine formation. Sulph-indigotate of soda was injected into the blood-stream, and the course of its excretion can be easily traced, as it colours the cells blue through which it passes, and is found then in the lumen of the tubules; it is found, not in the glomeruli, but only in the fibrillated epithelium of the convoluted tubules. It might be objected that this pigment is not a normal constituent of the urine, and that it is unfair to rest one's conclusions simply upon such experiments ; it is, unfortunately, not so easy to trace the course of the normal constituents of the urine as it is that of a blue pigment, but, though difficult, it has nevertheless been done. Salts of uric acid were traced to take the same course by Heidenhain as the sulph-indigotate; he also confirmed the previous observations of Bowman and v. Wittich, that in birds, which are animals that secrete a solid urine, crystals of urates could be actually seen within the epithelium cells. Nussbaum has shown finally that urea is also excreted by these cells. Nussbaum experimented on frogs and newts ; in these animals the glomeruli are supplied by the renal artery, and the tubules by the renal portal vein. By ligaturing the renal artery, the circulation to the glomeruli is cut off ; by ligaturing the renal portal vein, the circulation to the tubules is cut off. He found that sugar and peptone, injected into the circulation, pass into the urine of an intact kidney, but not when the renal artery is tied ; urea, on the other hand, is excreted only when the circulation among the tubules is intact ; he found also that water is excreted by the epithelium, so that water is excreted in two ways by the kidney—by the glomeruli and by the epithelial cells.

Influence of nerves on the secretion of urine.—As yet we are acquainted with no secretory nerves of the kidney ; we know merely the influence of the vaso-motor nerves on the filtration of the urine through the renal vessels. As a general rule, dilatation of the branches of the renal artery raises the pressure within the glomeruli, thus increasing the amount of water that passes through them ; and the more this dilatation is confined to the area of the renal artery alone, the greater is the amount of urine. Other circumstances that raise the pressure within the glomeruli also lead to an increase of urine ; these are an increase of the force or frequency of the heart's beat, an increase in the volume of the blood, and a constriction of the small vessels of areas other than that of the kidney. The opposite conditions, namely, those which diminish the pressure within the glomeruli, lead to a diminution of the amount of urine excreted.

Diuretics, however, may cause an increase of urine in one or more of several ways. There are some like digitalis, or squill, which do so by raising the blood-pressure; while others, like caffeine and potassium acetate, act on the secreting nerves, if such exist, or on the secreting cells of the kidney itself.[1]

In the foregoing brief *résumé* of the facts concerning the secretion of urine, the term *excretion* would perhaps have been more correct. We shall find, when we come to consider the constituents of the urine, that they are not actually formed in the kidney itself (as, for instance, the bile is formed by the liver), but they are mostly formed elsewhere, and the kidney is merely the place where they are eliminated from the body.

GENERAL CHARACTERS OF URINE

Quantity.—A man of average weight and height excretes 1400 to 1600 c.c. of urine daily. This is on an average about 50 ounces. This contains about 50 grammes (1½ oz.) of solids. A woman passes rather less. The quantity secreted may vary within certain limits in health, being increased by ingestion of much water, cold, moisture of the atmosphere, and by certain emotions; it is diminished by abstention from drinking, by copious sweating, diarrhœa, or vomiting.

The average secretion at different ages in children is given in the following table (Camerer)[2]:—

First day after birth	12 c.c.
Third „ „	23 „
Fifth „ „	35 „
Seventh „ „	51 „
Tenth „ „	61 „
At five months	1000 „

Urine should be collected in a tall glass vessel, capable of holding 3000 c.c., which should have a smooth-edged neck, accurately covered by a ground glass plate to exclude dust and avoid evaporation. The vessel, moreover, should be graduated, so that the amount may be easily read off. From the total quantity thus collected in the twenty-four hours, samples may be drawn off for examination.

Colour.—The colour of normal urine is that of a light sherry, but when concentrated, as in those who have perspired freely, it may be much darker, and when very watery, as after free potations, it may be much lighter in perfectly normal persons. In various abnormal conditions the colour varies considerably. The various urinary pigments in health and disease will be fully considered later. The following table gives in a concise way the chief variations in tint and their causes :—

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[1] See more fully Lauder Brunton, *Disorders of Digestion*, p. 866.

[2] *Zeit. Biol.* xiv. 388.

Colour	Cause of colouration	Pathological condition
Nearly colourless	Dilution, or diminution of normal pigments	Various nervous conditions: hydruria, diabetes insipidus, granular kidney
Dark yellow to brown red	Increase of normal, or occurrence of pathological pigments	Acute febrile diseases
Milky	Fat-globules	Chyluria
	Pus-corpuscles	Purulent disease of urinary tract
Orange	Excreted drugs	Santonin, chrysophanic acid[1]
Red or reddish	Unchanged hæmoglobin	Hæmorrhages or hæmoglobinuria
	Pigments in the food (logwood, madder, bilberries, fuchsin)	
Brown to brown-black	Hæmatin	Small hæmorrhages
	Methæmoglobin	Methæmoglobinuria
	Melanin	Melanotic sarcoma
	Hydrochinon and catechol	Carbolic acid poisoning

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[1] To distinguish these add caustic soda; this colours the urine red; agitate with amyl alcohol; the colour due to santonin passes into the alcohol, which in contact with atmospheric oxygen changes to yellow; the colour due to chrysophanic acid does not dissolve in amyl alcohol, or only in traces (Hoppe-Seyler, *Chem. Centralbl.* 1886, p. 746).

Colour	Cause of colouration	Pathological condition
Greenish yellow, greenish brown, approaching black	Bile-pigments	Jaundice
Dirty green or blue	A dark blue scum on surface with a blue deposit, due to excess of indigo-forming substances	Cholera, typhus. Seen especially when the urine is putrefying
Brown yellow to red brown, becoming blood-red on adding alkalis	Substances introduced into the organism with senna, rhubarb, and chelidonium	

Vogel[1] has drawn up a scale in which the colours are mapped out as pale yellow, bright yellow, yellow, reddish yellow, red, brown, and so on. . In my own experience, however, it is of little practical use.

The decolourisation of normal urine may be effected by the precipitation of the pigment (urobilin) by lead acetate and subsequent filtration. Worm-Müller's method has the advantage that it does not alter the composition of the urine, and consists in filling a filter or funnel with finely powdered animal charcoal made into a paste with the urine; the centre is scooped out, and through this charcoal filter the rest of the urine is allowed to drop into a collecting vessel.

Transparency or turbidity of urine.—Normal urine is either perfectly transparent, or contains a faint flocculent cloud of mucus. Old and decomposed urines are always turbid; this is the result of a deposit of certain salts and the growth of bacteria. Pathological turbid urines may contain chyle, blood, casts and renal epithelium, mucus, or pus. A turbid urine must always be allowed to settle before examination; both the sediment and the clear, supernatant fluid are then examined.

Odour.—Normal urine has an aromatic odour, said to be due to small quantities of phenylic, taurylic, and damoluric acids. Decomposing urine has an ammoniacal odour ; this is due to the conversion of part of the urea into ammonium carbonate ($CON_2H_4 + 2H_2O = (NH_4)_2CO_3$) brought about by the *torula ureæ*. This may occur after the urine is passed, or in cases of cystitis, in the bladder; in the cases where there is much decomposing blood or pus the smell may be putrid. In diabetes the urine often smells of acetone. Urine containing cystin smells at first like sweet briar, but afterwards becomes very offensive. Certain food and drugs taken internally give the urine their own or a characteristic odour. Instances of this are asparagus, garlic, copaiba, cubebs, sandal wood oil, tolu, turpentine, &c. The last named gives the urine a smell of violets.

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[1] Neubauer and Vogel's 'Guide to the Analysis of Urine,' *New Sydenham Soc. Trans.* 1863.

Reaction.—The reaction of normal urine is acid; this is not due to the presence of free acid, the uric acid in normal urine being all combined as urates. This is proved by the fact that sodium hyposulphite gives no precipitate (Voit, Huppert). Salkowski[1] leaves the question open, however, as to whether hippuric acid, which is present in small amount, is free or combined. It has, however, been settled recently by Brücke's[2] experiments with congo-red. One part of free hippuric acid in 55,000 of distilled water causes a solution of this reagent to become violet or inky, but urine gives no change of colour.

The acidity is due to acid sodium phosphate; this is derived from the basic sodium phosphate of the blood; the uric, hippuric, sulphuric, and carbonic acids of the urine take up part of the soda, leaving an acid salt.

The acidity of urine is increased by the internal administration of acids, a purely meat diet, and after prolonged muscular exertion.

Acid may be developed in stale urine (acid fermentation).

In certain pathological conditions free fatty acids may occur in the urine (lipaciduria).

Under certain circumstances the urine becomes less acid, or even alkaline. The most important of these is during digestion. Here there is a formation of free acid in the stomach, and a corresponding liberation of bases which pass into the urine, diminishing its acidity, or even making it alkaline. This is sometimes termed *the alkaline tide*; the opposite condition—*the acid tide*—occurs after a fast, for instance, before breakfast.

The following table gives a list of the chief circumstances under which the urine becomes alkaline :—

1. After a full meal, i.e. when the gastric juice is at work.
2. After the discharge of gastric juice in other ways, e.g. through a gastric fistula, or by vomiting.
3. After hot baths and free perspiration.
4. In herbivorous animals. (It becomes acid after fasting.)
5. In vegetarians: in these persons, as in herbivora, the food contains excess of alkaline salts, or acids like tartaric, citric, malic, succinic, &c. These acids are converted into carbonates, which, passing into the urine, give it an alkaline reaction.
6. After the medicinal administration of large quantities of alkaline carbonates, alkaline phosphates, or caustic alkalis.
7. From the decomposition of the urine either within the body, as in catarrh of the urinary tract, or after standing exposed to the air (alkaline fermentation). This alkalinity is due to the conversion of urea into ammonium carbonate.

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[1] *Lehre vom Harn*, Berlin, 1882, p. 15.

[2] *Monatsheft Chem.* viii. 95.

Specific gravity.—This should be taken in a sample of the twenty-four hours' urine, either by means of a good urinometer (fig. 9, p. 15) or more accurately by actual weighing. The specific gravity varies inversely with the quantity of urine passed; under normal conditions from 1015 to 1025; a specific gravity below 1010 should excite suspicion of hydruria; one over 1030 should excite suspicion of diabetes mellitus, a disease in which it may rise to 1050. The specific gravity has, however, been known to sink as low as 1002 (after large potations, *urina potus*), or to rise as high as 1035 or 1040 (after great sweating) in perfectly healthy persons. The concentration of the urine, and therefore its specific gravity, is raised in febrile conditions, and in the first stage of acute Bright's disease. Sugar being absent, the specific gravity varies as a rule with the percentage of urea.

Constituents of normal urine.—Hoppe-Seyler's[1] classification of the constituents of normal urine is the basis of the following :—

(1) Urea and related substances; uric acid, allantoin, oxalic acid, xanthine, guanine, creatinine, thio- (sulpho-) cyanic acid.

(2) Fatty and other non-nitrogenous substances; fatty acids of the series $C_nH_{2n}O_2$; oxalic, lactic, glycero-phosphoric acids; minute quantities of certain carbohydrates.

(3) Aromatic substances; the ethereal sulphates of phenol, cresol, pyrocatechin, indoxyl, and skatoxyl; hippuric acid; aromatic oxy-acids.

(4) Other organic substances; pigments, ferments, especially pepsin, mucus, humous substances; cynurenic and urocanic acids (in dogs).

(5) Inorganic salts; chlorides of sodium and potassium, potassium sulphate, sodium, calcium, and magnesium phosphates, silicic acid, ammonia compounds, calcium carbonate.

(6) Gases: nitrogen and carbonic acid.

Abnormal constituents of the urine.—In certain pathological conditions, there may be, in addition to the above, serum-albumin and other proteids, hæmoglobin, methæmoglobin, bile-pigments, bile-acids, abnormal urinary pigments, leucine and tyrosine, oxymandel acid, grape sugar, milk sugar, glycuronic acid, fats, lecithin, cholesterin, cystin; constituents derived from food or drugs; microscopic elements like blood-corpuscles, urinary casts, and renal epithelium.

Quantitative composition of human urine.—A large number of estimations have been made of the constituents of normal urine. I shall, however, merely quote the classical table from Parkes, and a recent analysis by Yvon and Berlioz of some of the more important substances present.

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[1] *Physiol. Chem.* p. 800.

Amounts of the urinary constituents passed in twenty-four hours (Parkes) :—

Constituents	By an average man of 66 kilos	Per kilo. of body-weight
Water	1500·00 grammes	23·000 grammes
Total solids	72·00 „	1·100 „
Urea	33·18 „	0·500 „
Uric acid	0·55 „	0·008 „
Hippuric acid	0·40 „	0·006 „
Creatinine	0·91 „	0·014 „
Pigment and other organic substances	10·00 „	0·151 „
Sulphuric acid	2·01 „	0·030 „
Phosphoric acid	3·16 „	0·048 „
Chlorine	7·00–8·00 „	0·126 „
Ammonia	0·77 „	—
Potassium	2·50 „	—
Sodium	11·09 „	—
Calcium	0·26 „	—
Magnesium	0·21 „	—

Mean composition of normal human urine (Yvon and Berlioz): —[1]

	Male	Female
Volume per diem	1360 c.c.	1100 c.c.
Specific gravity	1022 „	1021 „
Urea (per litre)	21·5 grammes	19·0 grammes
„ (per diem)	26·5 „	20·5 „
Uric acid (per litre)	0·5 „	0·55 „
„ (per diem)	0·6 „	0·57 „
Phosphoric acid (per litre)	2·5 „	2·4 „
„ „ (per diem)	3·2 „	2·6 „

The examination of the urine for normal constituents.—Before dealing in detail with the various constituents we have already enumerated, a general idea of the reactions of the principal constituents of the urine may be obtained from the following table :—

Substance sought	Test	Reaction	Remarks
Chlorides	Acidulate the urine with a few drops of nitric acid, and then add silver nitrate solution	A white precipitate of silver chloride, insoluble in nitric acid, soluble in ammonia	The principal chloride in urine is sodium chloride. Small quantities of potassium chloride are also present. The nitric acid used in the test holds phosphates in solution which would otherwise be precipitated

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[1] *Rev. med.* viii. 713; *Lancet*, vol. ii. 1888, p. 629.

Substance sought	Test	Reaction	Remarks
Sulphates . .	Acidulate with hydrochloric acid, and add solution of barium chloride	A white precipitate of barium sulphate, insoluble in nitric acid	The chief sulphate is potassium sulphate. Hydrochloric acid is added to prevent precipitation of phosphates and carbonates
Phosphates . .	Make urine alkaline with potash or ammonia, and warm	White flakes of earthy (Ca and Mg) phosphates are precipitated; soluble in acetic acid	In addition to the earthy phosphates, phosphates of sodium and potassium also occur, which vary in composition with the reaction of the urine. A deposit of phosphates may often occur in alkaline or neutral urine, or may come down on heating. This is soluble in acetic acid, and is thus distinguished from albumin
	Acidulate with nitric acid, add nitro-molybdate of ammonia, and warm	A yellow crystalline precipitate	
Calcium . . .	Acidulate with acetic acid, and add solution of ammonium oxalate	A white precipitate of calcium oxalate is formed; filter this off	
Magnesium . .	Treat the above filtrate with ammonia and a few drops of sodium phosphate	Ammonio-magnesium phosphate separates out	This is soluble in acetic acid
Sodium and potassium	Evaporate the urine to dryness; incinerate the residue; dissolve the ash in water; evaporate this down, and test by the flame reaction	Sodium gives a yellow, potassium a violet flame; potassium gives a yellow precipitate in neutral solutions with platinum chloride; sodium does not	
Ammonia . .	Heat the urine gently in a test-tube, holding a piece of red litmus paper over the mouth of it	The paper is turned blue, and regains its colour on gently warming it	Ammonia is only present in appreciable quantities in stale urine
Carbonic acid .	Place the urine in a tightly closed flask connected with a second flask, in which lime or baryta water is placed; the second flask is exhausted by an air-pump. Warm the urine gently	A white precipitate of calcium or barium carbonate, respectively is formed in the second flask	
	Hold a piece of moist blue litmus paper over the urine and warm	The paper is turned red, and regains its colour when dry	
Urea	Evaporate urine to a third of its bulk, and add nitric acid	Crystals of urea nitrate separate out	If the urine is albuminous render acid with acetic acid, boil and filter. Apply the tests to the filtrate
	Proceed as above with oxalic acid	Crystals of urea oxalate separate out	
	Add alkaline solution of sodium hypobromite	An evolution of bubbles of nitrogen takes place	

Substance sought	Test	Reaction	Remarks
Urea (*cont.*) . .	Add three parts of an aqueous solution of furfuraldehyde, a few drops of concentrated hydrochloric acid, and warm	A series of colours—yellow, green, violet, purple, red—is produced, settling finally into a brown resinous mass	
	Warm crystals of urea in a test-tube	Biuret is formed; add a few drops of potash and a drop of solution of copper sulphate; a rose-red colour is produced	
Uric acid . .	Add 5 c.c. of hydrochloric acid to 100 c.c. of urine; let it stand for twenty-four hours	Uric acid separates out in crystals, which fall to the bottom and stick to the sides of the vessel. Examine these microscopically	The crystals are coloured dark red by urinary pigment. The crystals may be collected, dissolved in soda, and again precipitated by hydrochloric acid
	Place the urine in a watch-glass, acidify with acetic acid, and place a cotton or linen thread in it; leave for twenty-four hours	The crystals collect along the thread. Examine microscopically, or apply murexide test	
	Murexide test.—The crystals are evaporated to dryness with nitric acid, and touched when cold with ammonia or potash	Ammonia turns the yellow deposit purplish red, owing to the formation of murexide which contains furfurate of ammonia [$C_8H_4(NH_4)N_5O_6$]. On adding potash after the ammonia the spot becomes purplish blue. If potash or soda alone is used, instead of ammonia, a violet colour appears, which disappears on heating	
	Schiff's test.—Dissolve the crystals in solution of sodium carbonate; drop this on to a filter-paper moistened with silver nitrate	A black spot of reduced silver appears	
	A solution of uric acid or urate warmed with copper sulphate and caustic potash	Produces a reddish precipitate of cuprous oxide	Hence urates may, when in excess in urine, be mistaken for sugar
Urates in urinary sediments	These dissolve on warming the urine		They are coloured pink by urinary pigment, forming the so-called lateritious deposit. The urates occurring in sediments are acid urate of soda, of potash, and ammonia
Urates in serpent's or bird's urine	The separation of uric acid from this is effected by finely powdering and dissolving in warm soda: filter: render the filtrate acid with hydrochloric acid. A white crystalline deposit of uric acid separates out		This urine is white powder when dry, creamy when fresh; it consists chiefly of ammonium urate

Substance sought	Test	Reaction	Remarks
ppuric acid .	250 c.c. of fresh urine are evaporated down to 25 c.c., and powdered gypsum added until it forms a thick paste; this is acidified with acetic acid and extracted with pure ether; distil the ether off from ethereal extract; dissolve residue in hot water, and filter	On cooling, hippuric acid crystallises out from the filtrate. The crystals may be purified from benzoic acid by petroleum ether, which dissolves H. acid, leaving B. acid insoluble. Distil off petroleum ether from extract, and dissolve residue in water as before	There is very little hippuric acid in human urine. It is abundant in the urine of horses and other herbivora
	Evaporate the urine with nitric acid, and heat the residue in a dry test-tube	A smell of oil of bitter almonds is given off	Benzoic acid also gives this reaction
atinine . .	Urine is evaporated to a quarter of its bulk, and after cooling poured off from residue, if any; this is precipitated by acetate of lead; excess of lead removed by H_2S; filter off lead sulphide; nearly neutralise filtrate with soda, and add concentrated mercuric chloride solution	A precipitate of creatinine with mercuric chloride is produced; this is suspended in water; a stream of H_2S is passed through it; and the lead sulphide filtered off. The filtrate is decolourised with animal charcoal, and evaporated to a small bulk. The remaining mass of creatinine hydrochloride is crystallised out twice from strong alcohol, and the HC removed by boiling with lead oxyhydrate	
	Take 250 c.c. of urine; add milk of lime and calcium chloride in excess to precipitate phosphates; filter, and evaporate to small bulk; to this add 50 c.c. absolute alcohol, and let mixture stand six hours. Add 10 to 15 drops of alcoholic solution of zinc chloride	Crystals (rosettes) of zinc chloride-creatinine form in the course of a day or so	

CHAPTER XXXIX

UREA, URIC ACID, AND ALLIED SUBSTANCES

THE end-products of nitrogenous metabolism are those substances by means of which nitrogen is excreted from the body. We have already, in connection with respiration, considered the chief end-product of carbon metabolism, namely, carbonic acid. The chief end-product of hydrogen metabolism is water, and this is got rid of by several channels—expired air, sweat, and urine. Nitrogen differs from carbon and hydrogen in not being burnt off as a simple oxide. Both the intake and the output of nitrogen, as well as the stages intermediate between these two ends of the series of changes, form a most complicated process. Nitrogen in a simple condition exists around us in the atmosphere ; yet we make no use of this abundant supply, but obtain it from the complex substances we call proteids ; and when we are getting rid of it, it is discharged as urea, uric acid, &c., which, though simple in comparison with proteids, are complex when compared with carbonic acid and water.

In mammals, urea is the most important of these end-products: it is the chief constituent of the urine. The same is true for fishes and amphibia. In birds and reptiles, and in many invertebrates, uric acid is the chief end-product of nitrogenous metabolism.

In human urine, Camerer[1] found that, out of every 100 grammes of nitrogen in it, 90 are on the average derived from urea, and the remaining 10 from other nitrogenous constituents (uric acid, &c.), which are often termed extractives. Pflüger and Bohland give a rather higher number; they state that on the average 13·4 per cent. of the nitrogen in urine is not combined as urea.[2] Bohland's method consists in first estimating the total nitrogen; the 'extractives' are then precipitated by hydrochloric and phosphotungstic acids; the nitrogen is estimated in this precipitate (extractive nitrogen) and also in the filtrate (urea-nitrogen). In addition, the amount of free ammonia is estimated ; the nitrogen in this, however, only amounts to 0·065 per cent. of the whole.

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[1] *Zeit. Biol.* xxiv. 306 ; xxvi. 84.

[2] In a more recent paper Bohland (*Pflüger's Archiv*, xlii. 30) gives a higher number still, 15·5.

UREA

Urea, or carbamide $CO(NH_2)_2$, is isomeric with ammonium cyanate $(NH_4)CNO$, from which it was first prepared synthetically by Wöhler (1828). It may also be prepared by the action of ammonia on carbonyl chloride, by the hydration of cyanamide, from ammonium carbonate, and by several other methods.

Preparation from urine.—Urea was first prepared in an impure condition from urine by Rouelle, then by Fourcroy and Vauquelin.[1] The following methods are those now generally adopted :—

(1) Evaporate the urine to a small bulk. Add strong, pure nitric acid in excess, keeping the mixture cool during the addition of the acid. Pour off the excess of fluid from the crystals of urea nitrate which are formed; strain through muslin, and press between filter paper. Add to the dry product barium carbonate in large excess, and mix thoroughly with sufficient methylated spirit to form a paste. Dry on a water-bath, and extract with alcohol; filter; evaporate the filtrate on the water-bath, and set aside to crystallise. The product may be decolourised by animal charcoal and purified by recrystallisation.

(2) The following method is well adapted for the preparation of microscopic specimens of urea and urea nitrate: Take 20 c.c. of urine; add 'baryta mixture' (two volumes of barium hydrate solution and one volume of barium nitrate solution, both saturated in the cold) until no further precipitate is produced; filter; evaporate the filtrate to a thick syrup on the water-bath, and extract with alcohol; pour off and filter the alcoholic extract; evaporate it to dryness on the water-bath, and take up the residue with water. Place a drop of the aqueous solution on a slide, and allow it to crystallise; crystals of urea separate out. Place another drop on another slide, and add a drop of nitric acid; crystals of urea nitrate separate out.

Properties of urea.—It is readily soluble in alcohol and in water, but not in ether. Its taste is saltish; it is odourless, and neutral to litmus paper. It crystallises in silky four-sided prisms with oblique ends, or in delicate white needles, when rapidly crystallised (fig. 91). When treated with nitric acid, nitrate of urea $(CON_2H_4.HNO_3)$ is formed; this crystallises in octahedra, lozenge-shaped tablets, or hexagons (fig. 92 *a*). When treated with oxalic acid, flat or prismatic crystals of oxalate of urea $(CON_2H_4.H_2C_2O_4 + H_2O)$ are formed (fig. 92 *b*). These crystals may be readily obtained in an impure form by adding the respective acids

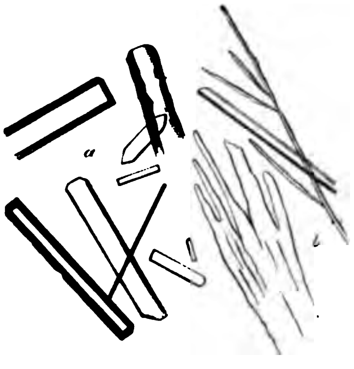

FIG. 91. Crystals of Urea; *a*, four-sided prisms; *b*, indefinite crystals, such as are usually formed from alcohol solutions.

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[1] *Ann de chim.* xxxii. 86.

to urine which has been concentrated to a third or a quarter of its bulk.

Other compounds of urea with acids have been also described; thus phosphate of urea ($CON_2H_4.H_3PO_4$) was said by Lehmann[1] to occur in small quantities in urine; a compound of urea with uronitrotoluolic acid, with the formula $C_{14}H_{19}N_3O_{10}$, was found by Jaffe[2] in dog's urine after the administration of orthonitrotoluol; the greater part of the urea in urine is, however, free.

Urea also forms compounds with salts; the most important of these is with mercuric nitrate; with this substance it forms a white precipitate, with the formula $CON_2H_4.Hg(NO_3)_2 + 3HgO$. This compound is important, as Liebig's volumetric process for the estimation of urea depends on its formation (*see* p. 810).

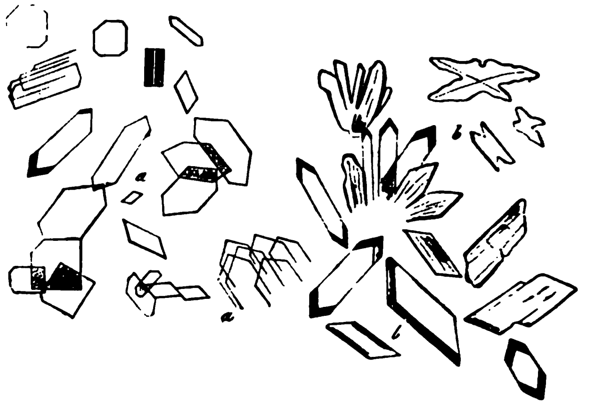

FIG. 92.—*a*, nitrate; *b*, oxalate of urea.

Drechsel[3] has described a compound of urea with palladium chloride ($PdCl_2 + 2CON_2H_4$).

There is also a crystalline compound of urea with sodium chloride ($CON_2H_4.NaCl + H_2O$), which may be obtained by evaporating to dryness a solution of these two substances, such as occurs, for instance, in ordinary urine.

Urea may be decomposed in various ways:—

(1) When heated to 150° to 170° it melts, and gives off ammonia: the substance which remains is termed biuret[4] ($2\underbrace{CON_2H_4}_{\text{[urea]}} - NH_3 = \underbrace{C_2O_2N_3H_5}_{\text{[biuret]}}$). Biuret with caustic potash and copper sulphate gives a characteristic rose-red solution. When biuret is heated it gives off ammonia, and cyanuric acid is left ($3\underbrace{C_2O_2N_3H_5}_{\text{[biuret]}} - 3NH_3 = 2\underbrace{C_3H_3N_3O_3}_{\text{[cyanuric acid]}}$). Cyanuric acid gives a violet solution with caustic potash and copper sulphate.

(2) By means of an organised ferment, the torula or micrococcus ureæ, which grows readily in stale urine, urea takes up water, and is converted into ammonium carbonate ($CON_2H_4 + 2H_2O = (NH_4)_2CO_3$).

(3) By means of nitrous acid, urea is broken up into carbonic acid, water, and nitrogen, $CON_2H_4 + N_2O_3 = CO_2 + 2H_2O + 2N_2$. This may be used as a test for urea: add fuming nitric acid to a solution of urea; an abundant evolution of gas bubbles takes place.

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[1] *Chem. Centralbl.* 1866, p. 1119.

[2] *Zeit. physiol. Chem.* ii. 50.

[3] *Journ. prakt. Chem.* N.F. xx. 469.

[4] *Poggendorf's Annalen*, lxx. [illegible]

(4) Chlorine water causes a somewhat similar decomposition ($CON_2H_4 + H_2O + 3Cl_2 = CO_2 + N_2 + 6HCl$).

(5) Hypochlorite or hypobromite of soda decomposes urea in the following way: ($CON_2H_4 + 3NaBrO = CO_2 + N_2 + 2H_2O + 3NaBr$). This reaction is important, as upon it depends one of the best methods of estimating the quantity of urea in urine (*see* p. 811).

Quantity of urea in urine.—The quantity of urea in urine varies a good deal, the chief cause of variation being the amount of proteid food ingested. In a man who is in a state of equilibrium, and on an ordinary mixed diet, the quantity of urea secreted daily is between 25 and 40 grammes, the average being 33 grammes (500 grains). On a diet poor in proteids it may sink to 15 to 20 grammes, and in a diet rich in proteids it may rise to 100 grammes per diem. We have seen that the concentration of the urine varies considerably in health, and thus the percentage amount of urea varies also. It may be roughly said that the quantity of urea in normal human urine is 2 per cent.; in dogs it may be 10 per cent.

Women secrete rather less than men; children absolutely less than adults, but in proportion to their weight more. Uhle gives the following table, which represents the amount of urea secreted in twenty-four hours per kilo. of body-weight at ages—

From 3–6 years . . .	about 1 gramme
„ 8–11 „ . . .	„ 0·8 „
„ 13–16 „ . . .	„ 0·4–0·6 „
Adults	0·37–0·6 „

The excretion of urea is usually at its maximum three hours after a meal, especially after a meal rich in proteids. The quantity of urea does not, however, necessarily depend on increased production of urea; a long-continued increase in urea indicates increased tissue-metabolism, but a temporary increase may be merely produced by an increase of the urinary secretion, by which the urea collected in the body is quickly passed off. In the same way diminished excretion of urea may be due either to diminished metabolism, or to retention of urea in the body, as in uræmia. These considerations are especially useful in determining the influence of food on urea excretion. In the first place the urea does not come direct from the food; the food must be first assimilated and become part of the body before it can break down to form urea.[1] The urea is increased by food, first because food stimulates the tissues to activity, and so metabolism is increased; and, secondly, stimulates the kidneys to activity, and so waste accumulated products are got rid of.

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[1] An exception to this rule is probably to be found in the case of the amido-acids, especially leucine. (*See* further under Metabolism, p. 845.)

Camerer[1] carried out experiments on four persons, each of whom partook of only one meat meal in the twenty-four hours, and whose urine was collected at intervals of three hours; these samples were analysed separately, the urea-nitrogen and the extractive nitrogen (i.e. total nitrogen *minus* urea-nitrogen) being estimated. The increase in both kinds of nitrogen commenced almost immediately after the meal, the urea-nitrogen reaching its maximum in from seven to ten hours, while that derived from the extractives was greatest in the first four hours after the meal. The quantity of urine was smallest during the first four hours, and greatest seven to ten hours after the meal; the least concentrated urine was that accompanied by the secretion of the greatest total amount of urea.

A large number of observations on the influence of other varying conditions on the amount of urea excreted, have been recorded, and may be tabulated as follows :—

Circumstances producing	
An increase of urea	A decrease of urea
Administration of—	Administration of—
Dilute sulphuric acid (Kurtz[2]), potassium chloride (Dehn[3]), ammonium salts, especially with food,[4] small doses of phosphorus, arsenic, antimony, morphia, codeia (Gäthgens[5]), large doses of quinine (Oppenheim[6]).	Small doses of quinine (Oppenheim).
Poisoning by— Phosphorus (Storch,[7] Bauer[8]), arsenic (Kossel[9]).	
Application of cold to the skin (Voit[10]).	
Hot baths (Schleich[11]).	
Increase of oxygen inspired (Fränkel[12]).	
Excessive muscular work (*see* p. 436).	
Diseases :—	Diseases :—
At the commencement of acute febrile diseases, up to the acme of the fever.	During the sinking of the fever
During the paroxysms of intermittent fever (ague).	
	In most chronic and debilitating diseases (anaemia, syphilis, phthisis, dropsical affections, &c.)
	Towards the fatal termination of most diseases (5 to 6 grms. daily).
	In uraemia; the secretion may entirely cease.
In diabetes.	In diabetic coma.
	In all degenerative changes of the liver, especially in acute yellow atrophy.

The formation of urea.—The formation of urea occurs through the whole of healthy extra-uterine life. It is also formed in the fœtus, but there its place is, to a large extent, taken by another substance called *allantoin*.

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[1] *Zeit. Biol.* xxiv. 806. [2] Kurtz, *Diss.* Dorpat, 1874. [3] *Diss.* Rostock, [illegible]

[4] Hallervorden, *Arch. ex. Path. u. Pharm.* x. 124; Feder and Voit, *Zeit. Biol.* xvi. [illegible]

[5] Quoted by MacMunn, *Clin. Chem. of Urine*, p. 86. [6] *Pflüger's Archiv*, xxiii. 446.

[7] *Der* [illegible] Copenhagen, 1875; *Arch. f. klin. Med.* 1867, vol. [illegible]

[8] [illegible] *Pathol.* v. 128. [10] *Zeit. Biol.* xiv. [illegible]

[illegible] *Arch. f. pathol. Anat.* lxvii. 1; lxxi. 117.

The important questions in relation to the formation of urea are, first, where is it formed? and secondly, from what is it formed?

Where is urea formed? The older authors considered that it was formed in the kidneys, just as they also erroneously considered that carbonic acid was formed in the lungs. Prévost and Dumas[1] were the first to show that after complete extirpation of the kidneys, the formation of urea goes on, and it accumulates in the blood and tissues. Similarly in those cases of disease, in which the kidneys cease work, urea still continues to be formed and accumulates in the body. If, then, the kidneys are not specially the seat of formation of urea, where is this special seat, or is there any special seat? If we look to the most abundant tissue of the body the muscles—we find urea absent, or nearly so; there can, however, be no doubt that some intermediate steps in the process takes place in the muscles.[2] In the muscles we find the place of urea taken by creatine; some of this is undoubtedly excreted as creatinine. Whether some is further changed into urea is a matter of doubt, and has already been discussed in connection with muscle (pp. 419, 439).

The liver is now generally supposed to be the chief place where urea is formed; this view was originally put forward by Meissner;[3] but, although contradicted by Gschleidlen,[4] Munk,[5] and Pekelharing,[6] it is supported by the more recent experiments of Brouardel,[7] Roster,[8] Schrœder,[9] and Minkowski.[9] It is, however, very probable that other cellular organs like the spleen, lymphatic and secreting glands participate in the formation of urea. The urea passes into the blood, is carried to the kidneys, and is there excreted.

The facts of pathology point very strongly in support of the theory that urea is formed in the liver. Diabetes is sometimes a disease of the liver in which the metabolism of its cells is much increased, leading to an abundant formation of sugar which passes into the blood and urine; and in these cases the urea is also increased.

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[1] *Ann. de chim. et de phys.* xxiii. 90. This observation has been since confirmed by many observers, e.g. Tiedemann and Mitscherlich, *Poggendorf's Annalen*, xxxi. 808; Marchand, *Journ. prakt. Chem.* xxi. 260.

[2] Selachian fishes form an exception to this rule. The kidneys appear to be sluggish and urea accumulates in the blood to an enormous extent (2·6 per cent.); the muscles contain 1·9 and the liver 1·8 per cent. of urea (*Zeit. physiol. Chem.* xiv. 576).

[3] *Zeit. rat. Med.* N.F. xxxi. 284.

[4] *Studien ü. d. Ursprung des Harnstoffs*, Leipzig, 1871.

[5] *Pflüger's Archiv*, ii. 100.

[6] *Ibid.* p. 608.

[7] *Arch. de physiol. norm. et pathol.* (2), iii. 373, 551.

Italian paper quoted by Hoppe-Seyler, *Physiol. Chem.* p. 807.

[9] *See* pp. 727, 735.

obtained, but the intermediate step in the process is not the formation of alloxan, but of another somewhat similar substance called allantoin; this process is interesting, as allantoin is in fœtal life one of the products of nitrogenous metabolism, and it is thus possible that some sort of change, such as can be produced artificially, occurs in embryonic life.

Uric acid when oxidised with potassium permanganate (care being taken that the temperature does not rise) takes up water and oxygen, forming allantoin and carbonic acid:

$$\underset{\text{[uric acid]}}{2C_5H_4N_4O_2} + 2H_2O + O_2 = \underset{\text{[allantoin]}}{2C_4H_6N_4O_3} + 2CO_2.$$

The allantoin crystallises out in about twenty-four hours. By subjecting allantoin to the action of baryta-water, hydrolysis and oxidation again take place, and urea and oxalic acid are formed:

$$\underset{\text{[allantoin]}}{2C_4H_6N_4O_3} + 4H_2O + O_2 = \underset{\text{[urea]}}{4CON_2H_4} + \underset{\text{[oxalic acid]}}{2C_2H_2O_4}.$$

(5) The following decompositions are interesting, as the murexide test is the chief characteristic test for uric acid.

By oxidation with nitric acid, alloxan and urea are formed:

$$2C_5H_4N_4O_3 + 2H_2O + O_2 = 2C_4H_2N_2O_4 + 2CON_2H_4.$$

By heating or by electrolysis, alloxan splits into alloxantin, parabanic acid, and carbonic acid:

$$\underset{\text{[alloxan]}}{3C_4H_2N_2O_4} = \underset{\text{[alloxantin]}}{C_8H_4N_4O_7} + \underset{\text{[parabanic acid]}}{C_3H_2N_2O_3} + CO_2;$$

and on treating alloxantin with ammonia the purple colour due to murexide or purpurate of ammonia appears:

$$\underset{\text{[alloxantin]}}{C_8H_4N_4O_7} + 2NH_3 = \underset{\text{[murexide]}}{C_8H_8N_6O_6} + H_2O.$$

Compounds of uric acid.—Uric acid is dibasic, and thus there are two classes of urates, the normal urates and the acid urates. A normal urate is one in which two atoms of the hydrogen are replaced by two atoms of a monad metal like sodium ($C_5H_2Na_2N_4O_3$): an acid urate is one in which only one atom of hydrogen is thus replaced; the acid urate of sodium has, therefore, the formula $C_5H_3NaN_4O_3$.

The urates of the alkalis are those which are obtainable from the urine. The most abundant urate obtained from human urine is the normal sodium urate; small quantities of those of potassium and calcium also occur. The acid ammonium urate is the chief constituent separable from the excrement of birds and reptiles (fig. 95).

The urates, like uric acid, are insoluble substances, and hence if excess occurs in the urine, they will be precipitated when the urine cools, after it is passed. This will especially occur if what is called the acid fermentation takes place, the acid urate of sodium being much more insoluble than the normal salt. The reaction that occurs may be thus represented:

$$\underset{\text{[normal sodium urate]}}{2C_5H_2Na_2N_4O_3} + H_2O + CO_2 = \underset{\text{[acid sodium urate]}}{2C_5H_3NaN_4O_3} + \underset{\text{[sodium carbonate]}}{Na_2CO_3}.$$

The acid sodium urate (fig. 94) is, indeed, the chief component of the pinkish deposit of urates (often called lithates) that occurs in concentrated, cold, acid urine. This deposit, sometimes called the *lateritious* deposit, from its resemblance to brick-dust, is generally amorphous, or only partly crystalline. The pink colour is derived from the urinary pigment, and is called *uroerythrin*. This deposit can be readily distinguished from other urinary sediments by the fact that it dissolves upon warming the urine to the temperature of the body. A certain amount of calcium oxalate crystals (octahedra) will often be found mixed with the urates. The close relationship of uric and oxalic acids is apparent from the formulæ on pp. 729, 730.

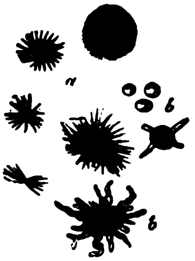

FIG. 94.—Acid Sodium Urate.

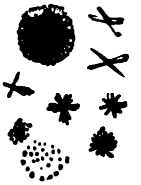

FIG. 95. Acid Ammonium Urate.

The following table [1] gives concisely certain facts relating to the urates : —

Urates	Formulæ	Solubility in water	Deposited as
Acid ammonium urate .	$C_5H_3N_4O_3.(NH_4)$	1 in 1600	Amorphous or spiked globular masses
Normal sodium ,, .	$C_5H_2N_4O_3.Na_2$	1 ,, 77	Nodular masses
Acid sodium ,, .	$C_5H_3N_4O_3.Na$	1 ,, 1200	Amorphous; rarely crystalline
Normal potassium ,, .	$C_5H_2N_4O_3.K_2$	1 ,, 44	Amorphous: or in fine needles
Acid ,, ,, .	$C_5H_3N_4O_3.K$	1 ,, 800	---
Normal calcium ,, .	$C_5H_2N_4O_3.Ca$	1 ,, 1500	Fine granules
Acid ,, ,, .	$(C_5H_3N_4O_3)_2Ca$	1 ,, 600	Amorphous; or in fine needles
Acid lithium ,, .	$C_5H_3N_4O_3Li$	1 ,, 60	Ditto

The greater solubility of potassium and lithium urates has led to the administration of potash and lithia in cases where uric acid or urates are in excess in the urine, and it is desired to dissolve them up. The calcium urates occur in mere traces in urine, but have been found in gouty deposits, in addition to sodium urates (*see* p. 510).

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[1] *Ralfe's Diseases of the Kidneys*, 1885, p. 81.

Sir W. Roberts[1] has recently investigated the urates of the urine, and the following is a *résumé* of his paper:

'The presence of uric acid in human urine is somewhat anomalous. As a vehicle for the elimination of nitrogen, it is not needed. Its place is taken by urea, which, by its easy solubility, is better adapted to the liquid urine of mammals. Perhaps uric acid is a vestigial remnant in mammalian descent. But, although physiologically insignificant, uric acid is pathologically the most prominent component of the urine, this being chiefly due to its tendency to form concretions.

'All acid urines tend inevitably to deposit their uric acid sooner or later. The time of onset of precipitation varies from a few hours to five or six days, or even longer. The inference from this is that pathological gravel is due to an exaggeration of conditions which exist in a less pronounced degree in health. To get at an explanation of this spontaneous precipitation it is necessary to examine the states of combination of uric acid in urine.

'Uric acid ($C_5H_4N_4O_3 = H_2U$) is a bibasic acid, and forms two regular orders of salts, namely, *neutral* or *normal urates* (M_2U) and *acid urates* or *biurates* ($MH\bar{U}$).[2] But in addition to these it forms a series of hyperacid combinations, first discovered by Bence Jones, and termed by him *quadrurates* ($MH\bar{U}.H_2\bar{U}$). The neutral urates are never found in the animal body, and are only known as laboratory products. The biurates are only encountered pathologically as gouty concretions. The quadrurates, on the other hand, are especially the physiological salts of uric acid. They constitute the exclusive combination in which uric acid exists in solution in normal urine, and they become visible sometimes as the amorphous urate sediment. The urinary excretion of birds and serpents is composed exclusively of quadrurates. The quadrurates can, moreover, be formed artificially under conditions which prevail in the animal body. The special and characteristic reaction of the quadrurates is that they are immediately decomposed by water into free uric acid and biurates.

'They exist in acid urine in the presence of water and of superphosphates. These conditions necessarily involve the ultimate liberation and precipitation of uric acid. The first step is the breaking up of the quadrurate by the water of the urine into free uric acid and biurate according to the following equation:—

$$\underset{\text{[quadrurate]}}{(MH\bar{U}.H_2\bar{U})} + H_2O = \underset{\text{[free uric acid]}}{(H_2U)} + \underset{\text{[biurate]}}{(MH\bar{U})}.$$

'This explains the liberation of half the uric acid. But the biurate thus formed is forthwith changed in the presence of superphosphates into quadrurate. Thus :—

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[1] *Proc. Med. Chir. Soc.* 1890, p. 85.

[2] In these formulæ, the symbol M represents a monad metal, and the symbol U, the radicle $C_5H_2N_4O_3$.

$$\underset{\text{[biurate]}}{2(MH\bar{U})}+\underset{\text{[superphosphate]}}{(MH_2PO_4)}=\underset{\text{[quadrurate]}}{(MH\bar{U}.H_2\bar{U})}+\underset{\text{[dimetallic phosphate]}}{(M_2HPO_4)}.$$

By these alternating reactions all the uric acid is at length set free.

'Seeing that uric acid exists in acid urine (that is, for some sixteen hours out of the twenty-four) amid conditions which, if the quadrurates stood alone and uncontrolled, would lead to its immediate precipitation, and yet that in the normal course no such early precipitation occurs, it is obvious that the urine must contain certain ingredients which inhibit or greatly retard its water from breaking up the quadrurates. These inhibitory ingredients consist chiefly of (1) the mineral salts, (2) the pigments of the urine.

'The conditions of the urine which tend to accelerate the precipitation of uric acid, as in the formation of concretions and deposits, are (1) high acidity, (2) poverty in mineral salts, (3) low pigmentation, (4) high percentage of uric acid. The converse conditions tend to retard precipitation. On the interaction of these factors the occurrence or non-occurrence of uric acid gravel appears to depend, and probably the most important of these factors is the grade of acidity.'

Quantity of uric acid in the urine.—The quantity excreted by an adult man varies from seven to ten grains (0·5 to 0·75 gramme); during hunger it sinks to four grains.[1] Parkes gives the average percentage of uric acid in human urine as 0·03 to 0·05, and the proportion of urea to uric acid as 45 : 1.

Haig[2] states that the excretion of uric acid is much affected by food, being greatest during the 'alkaline tide' that follows a meal. He regards this as chiefly a washing out of the uric acid accumulated in the liver and spleen in the period between meals (acid tide); but there can be little doubt that, as the activity of these organs is increased after food, increased metabolism in general and increased production of uric acid in particular also take place.

Certain morbid conditions increase, certain others diminish, the excretion of uric acid; the appearance of a sediment of urates, however, does not necessarily mean an increased formation of urates; it may be due to increased concentration of the urine. A deposit of urates occurs when the urine is concentrated, as after violent exercise, and consequent profuse sweating; it also occurs in the concentrated urine of fever; after indigestion, inflammation of joints, in certain heart and lung affections, in cirrhosis of the liver, and occasionally in catarrh of the bladder where the acid fermentation is taking place. Free uric acid in crystals occurs in the urine of persons with a uric acid diathesis; a condition allied to

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[1] Estimations of uric acid secreted in health have been made by Becquerel (*Gmelin's Handb.* viii. 827), H. Ranke (*Ausscheidung der Harnsäure beim Menschen*, München, 1858), Neubauer (*Text-book*, p. 881), J. Ranke (*Grundzüge der Physiol.* Leipzig), Beneke (*Pathol. des Stoffwechsels*, Berlin, 1874), and many others.

[2] *Journ. of Physiol.* viii. 211.

obtained, but the intermediate step in the process is not the formation of alloxan, but of another somewhat similar substance called allantoin; this process is interesting, as allantoin is in fœtal life one of the products of nitrogenous metabolism, and it is thus possible that some sort of change, such as can be produced artificially, occurs in embryonic life.

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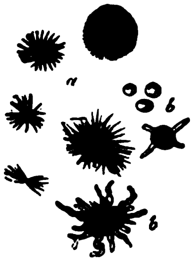
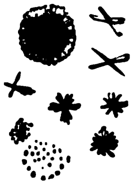


FIG. 94.—Acid Sodium Urate.



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Acid sodium .. .	$C_5H_3N_4O_3.Na$	1 „ 1200	Amorphous: rarely crystalline
Normal potassium „ .	$C_5H_2N_4O_3.K_2$	1 „ 44	Amorphous: or in fine needles
Acid „ „ .	$C_5H_3N_4O_3.K$	1 „ 800	-
Normal calcium „ .	$C_5H_2N_4O_3.Ca$	1 „ 1500	Fine granules
Acid „ „ .	$(C_5H_3N_4O_3)_2Ca$	1 „ 600	Amorphous: or in fine needles
Acid lithium „ .	$C_5H_3N_4O_3Li$	1 „ 60	Ditto

The greater solubility of potassium and lithium urates has led to the administration of potash and lithia in cases where uric acid or urates are in excess in the urine, and it is desired to dissolve them up. The calcium urates occur in mere traces in urine, but have been found in gouty deposits, in addition to sodium urates (*see* p. 510).

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[1] *Ralfe's Diseases of the Kidneys*, 1885, p. 81.

of their urine; it has also been found in the organs of many invertebrates that correspond to the vertebrate kidney; e.g. the green glands of crustacea,[1] the Malpighian tubes of insects, and the nephridia of certain molluscs.[2] It is more abundant in carnivorous animals than in man.[3] In herbivora, though replaced to some extent by hippuric acid, it is, nevertheless, fairly abundant.[4]

Preparation from urine.—If 5 c.c. of hydrochloric acid be added to 100 c.c. of urine, and the mixture be allowed to stand for twelve to twenty-four hours, crystals of uric acid separate out, and either fall to the bottom of the containing vessel, or adhere to its sides. These crystals are coloured dark-red by the urinary pigment, and may be obtained fairly free from it by repeated solution in caustic soda or potash, and re-precipitation by hydrochloric acid.

If, however, one wishes to prepare pure uric acid, the solid urine of a reptile or bird, which consists principally of the acid ammonium salt, should be selected; one has not then to separate any pigment. It is boiled with 10 per cent. caustic soda or ammonia; diluted, and then allowed to stand. The clear fluid is decanted, and poured into a large excess of water to which 10 per cent. of hydrochloric acid has been added; after twenty-four hours, crystals of uric acid are deposited. These may be purified by washing, re-solution in soda, and re-precipitation by acid.

Properties of uric acid.—Pure uric acid crystallises in colourless rhombic rectangular plates, or in rectangular prisms. In striking contrast to urea, it is a most insoluble substance, requiring for its solution 1,900 parts of hot and 15,000 parts of cold water. It is very slightly soluble in alcohol and ether. The urates are also very insoluble substances.

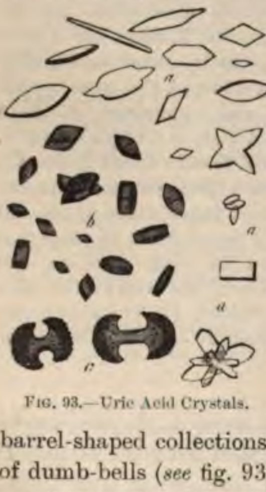

FIG. 93.—Uric Acid Crystals.

The precipitate of uric acid obtained in cases of gravel, and also that produced by the decomposition of urates which occurs when acid is added to the urine, is always deeply tinged red or brown by urinary pigment, the deposit having a cayenne pepper-like appearance. The forms which uric acid assumes under these circumstances are very various, the most frequent being the whetstone shape; there are also sheaf-like or barrel-shaped collections of needles; some of the bundles take the form of dumb-bells (*see* fig. 93).

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[1] Griffiths, *Chem. News*, li. 121.

[2] MacMunn, *Journ. of Physiol.* vii. 128.

[3] But not universally. *See* Sanarelli, *Chem. Centralbl.* 1887, p. 804.

[4] Mittelbach, *Zeit. physiol. Chem.* xii. 463.

In herbivora these compounds are more abundant in the urine than in flesh feeders like dogs, or those who live on a mixed diet like man. They are, however, found in small quantities in the urine of all animals.

They appear to have one or both of two origins : first, from the aromatic substances in the food ; hence their greater abundance in the urine of herbivora ; secondly, they arise in the intestine as a result of putrefaction. They are absorbed from the intestine, pass into the blood-stream, and are eliminated in combination with potassium as ethereal sulphates in the urine. The synthesis probably occurs in the liver (Baumann).

In animals like dogs and men, whose food contains little or nothing of an aromatic nature, the origin of the ethereal sulphates appears to be wholly under the second of these headings. If putrefaction be entirely stopped in the alimentary canal, these bodies completely disappear from the urine. Putrefaction can be put a stop to in the intestine of dogs by inanition *plus* the administration of large doses of calomel (Baumann, Morax[1]) or iodoform (Morax). In men, however, it is not possible to give doses of these drugs sufficiently large for the purpose. Baumann, however, was fortunate enough to make observations on a patient who had a fistula in the upper part of the intestine, and whose intestine below this was functionless ; putrefactive processes did not occur, and these salts were absent from the urine.

A large number of analyses have been made as to the relation of the ethereal sulphates to the total sulphates of the urine in man, and in round numbers the normal proportion may be stated as 1 : 10. The method employed in this analysis will be described in the chapter on Quantitative Analysis of Urine.

In morbid urines the same subject has been investigated by numerous analysts, among whom may be particularly mentioned G. Hoppe-Seyler[2] in Germany, and J. S. Haldane[3] in this country. It is found that in those diseases in which putrefaction in the intestines, or elsewhere in the body, is increased, the proportion of ethereal sulphates rises. G. Hoppe-Seyler's results may be summarised as follows:—

(1) Deficient absorption of the normal products of digestion, such as occurs in peritonitis and tubercular disease of the intestine, leads to an increase of the ethereal sulphates in the urine, because the products of digestion undergo putrefactive changes, and the putrefactive products are absorbed.

(2) Diseases of the stomach, in which the food lies in the stomach a long time and undergoes fermentative changes, always lead to an increase of the ethereal sulphates in the urine.

(3) Simple constipation and typhoid fever do not produce this result.

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[1] *Zeit. physiol. Chem.* x. 318.

[2] *Ibid.* xii. 1.

[3] *Journ. of Physiol.* ix. 218.

(4) Putrefactive processes outside the alimentary canal, putrid cystitis, putrid abscesses, putrid peritonitis, &c. have the same result as putrefactive processes within the intestine. The amount of the ethereal sulphates is, moreover, in all cases proportional to the severity of the putrefaction, and is increased by the retention and diminished by the discharge of putrid matter; as, for instance, on opening the abscess.

It has by these and other observations been conclusively shown that the best criterion of the occurrence and amount of putrefaction in the body is the relation of the ethereal sulphates to the total sulphates.

Brieger's method of estimating putrefactive change was by estimating the total quantity of phenol in the distillate from acid urine as tribromophenol. He found that it was increased in septic conditions.[1] This method is, however, open to two objections: first, it only takes into account one of the many products of putrefaction; and, secondly, as an analytical method, it is open to criticism. This latter point has been especially insisted on by Haldane, who found that the more concentrated the urine is, the greater is the quantity of tribromophenol to be obtained from it. The non-recognition of this source of error has led Brieger to classify scarlet fever and diphtheria with pyæmia, erysipelas, and other putrefaction processes, whereas the increase of phenol noted in these cases is merely due to the greater concentration of the urine that occurs. Haldane found, by the more accurate method of estimating the relation of ethereal sulphates to total sulphates, that the proportion is not increased, but if anything somewhat diminished; hence there are no grounds for classifying scarlet fever and diphtheria as putrefaction diseases except when complicated by the formation of putrid abscesses.

We must now pass from general considerations to consider the individual members of the group.

Phenol-sulphate of potassium.—The formula for carbolic acid or phenol is C_6H_6O. This was first found to be one of the products of intestinal putrefaction by Baumann.[2] This is absorbed and excreted as the phenol-sulphate of potassium ($C_6H_5O.SO_3K$) in the urine. Some of this sulphate also comes from tyrosine, which passes through the stages of paracresol and paraoxybenzoic acid before it is converted into the phenol salt (Baumann[2]).

Phenol may be obtained from urine by distilling it with sulphuric or hydrochloric acid. This breaks up the phenol sulphate, and phenol passes into the distillate, where it may be recognised by the yellow precipitate of tribromophenol which it gives with bromine water, or by the various colour-reactions already enumerated (p. 77).

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[1] *Centralbl. med. Wiss.* 1878, No. 30; *Zeit. physiol. Chem.* ii. 241.

[2] *Zeit. physiol. Chem.* i. 60; iii. 250. The view here advanced by Baumann as to the fate of tyrosine is not universally accepted; thus Blendermann (*Abst. Journal Chem. Soc.* 1883, p. 876) and Jaffe (*Zeit. physiol. Chem.* vii.) by feeding animals with tyrosine found no increase of aromatic or any other substances in the urine. Intravenous injection of tyrosine was followed by a similar negative result (Cohn, *Ibid.* xiv. 189).

After the medicinal or surgical use of carbolic acid the amount of the phenol-sulphate in the urine is increased; two substances are also formed by the breaking up of carbolic acid, called pyrocatechin and hydroquinon. These become in alkaline urine dark brown on exposure to the atmospheric oxygen, and it is this that produces the well-known colour of the urine in so-called 'carboluria.'

Cresol sulphate of potassium.—This is not so abundant as the phenol-salt, but may be obtained in the following way from the urine of an herbivorous animal like the horse: 3 or 4 litres are evaporated to a syrup; this is extracted with absolute alcohol, filtered, and the filtrate precipitated with an alcoholic solution of oxalic acid; the precipitate is removed by filtration, and the filtrate made slightly alkaline with caustic potash. This produces a precipitate, which is filtered off; the filtrate is evaporated to a thin syrup, which is then kept at a temperature of 0° C. Leafy crystals of the potassium cresol sulphate separate out, and may be purified by recrystallisation out of absolute alcohol (Krukenberg[1]). The formula for cresol is $C_7H_7(HO)$; that for the potassium sulphate of cresol is $C_7H_7O.SO_3K$. Cresol, like phenol, gives a red colour with Millon's reagent, but no violet colour with ferric chloride.

Of the three isomerides called cresol, that known as paracresol is the most abundant; ortho- and metacresol also combined as sulphates are present in mere traces. (The meaning of the prefixes ortho-, meta-, and para- in connection with aromatic substances is explained on p. 76.)

Some of the paracresol formed in the intestine is further changed into phenol and excreted as the phenol-sulphate of potassium (Baumann).

Catechol-sulphate of potassium.—Catechol or pyrocatechin, $C_6H_6O_2$, has two isomerides, named hydroquinon and resorcin. These were first found as ethereal sulphates in the urine of dogs after they had been fed on dihydroxyl combinations of benzene.[2] This salt of pyrocatechin has since been found normally in traces in human urine, especially in children,[3] but more abundantly in that of the horse. It becomes darker when the urine putrefies. This, however, is only apparent when catechol appears in abnormally large quantities (*see* Carboluria, Alkaptonuria). Protocatechuic acid both free and as an ethereal sulphate is also present (Baumann,[4] Preusse[5]).

Indoxyl-sulphate of potassium.—The parent of this substance, named indole (C_8H_7N), is formed in the intestine; indoxyl, a radicle derived from this, has the formula C_8H_6NO; this united with SO_3K forms the indoxyl-sulphate which is found in the urine ($C_8H_6NO.SO_3K$). This substance forms white glancing tablets and plates, easily soluble in water, less so in alcohol. By oxidation, indigo-blue is formed from it $(\underset{\text{[indox. sulph. of potassium]}}{2C_8H_6KNSO_4}+O_2=\underset{\text{[indigo-blue]}}{2C_8H_5NO}+\underset{\text{[pot. hyd. sulphate]}}{2HKSO_4})$.

The indoxyl-sulphate of potassium has received the unfortunate name of indican, under the mistaken idea that it is identical with plant indican. This latter substance is a glucoside, and only resembles the indican of urine, in that one of its decomposition products is indigo blue (*see* pp. 78, 79). The following methods have been devised for obtaining indigo from urine :—

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[1] *Grundriss der med. chem. Anat.* p. 87.

[2] Baumann and Herter, *Zeit. physiol. Chem.* i. 244; ii. 335.

[3] Ebstein and Müller, *Arch. path. Anat.* lxii. 554; Furbringer *Berlin. klin. Woch.* 1875, Nos. 24 and 28; Fleischer, *Ibid.* Nos. 39 and 40.

[4] *Pflüger's Archiv*, xii. 63; xiii. 16.

[5] *Zeit. physiol. Chem.* ii. 329.

(1) Jaffe's method[1] :—Equal parts of urine and hydrochloric acid are mixed; to this mixture a few drops of a saturated solution of 'bleaching powder' are added cautiously till the maximum of blue colour appears. The mixture is then agitated with chloroform, which takes up the blue pigment ; on evaporating off the chloroform the indigo is left. If this is weighed an approximate quantitative estimation of the amount of indigo in the volume of urine originally taken can be made. Albumin, if present, must be separated before performing this test, as it develops a blue colour with hydrochloric acid.

(2) MacMunn's method[2] :—Equal parts of urine and hydrochloric acid with a few drops of nitric acid are boiled together, cooled, and agitated with chloroform. The chloroform is generally violet, and shows an absorption band before D, due to indigo blue, and another after D, due to indigo red (*see* fig. 97, spectrum 4, p. 748). This method is preferable to Jaffe's, as 'bleaching powder' destroys small quantities of indigo.

The quantity of indigo in the urine of starving dogs was found by Salkowski[3] to be 4 to 5 milligr. in three days. After abundant meat meals it rose to 16 to 17 milligr. *per diem.* In 1500 c.c. of normal human urine, Jaffe found from 4 to 19 milligr. of indigo. Horse's urine contains twenty-three times as much. The greater abundance of indigo, like that of other aromatic substances in the urine of herbivora, depends on the diet. It is absent in the urine of new-born children.[4]

When indol is injected under the skin, or given by the stomach, it appears in the urine as indoxyl sulphate of potassium (Jaffe,[5] Nencki and Masson,[6] Christiani[7])

This salt is also increased in the urine in intestinal obstruction, peritonitis, typhus, cholera, cancer of the liver, long-standing suppuration, and Addison's disease. No doubt in many of these cases this is due to increased absorption of putrefactive products. Many aromatic drugs, like turpentine, oil of bitter almonds, and creosote, also increase its amount in the urine.

Sometimes urine shows when decomposing a bluish red pellicle of microscopic crystals of indigo blue and red, owing to the decomposition of the indoxyl-sulphate (Hill-Hassal, Stirling). A calculus composed of such crystals has been once described (Ord).

Skatoxyl - sulphate of potassium. — Skatole is methyl-indole $C_8H_6(CH_3)N$. Like indole, it is formed by the putrefaction of proteids in the intestine; some of it is absorbed, and passes into the urine as the skatoxyl-sulphate of potassium ($C_9H_8NO.SO_3K$). It is rather more abundant in human urine than the indoxyl-salt.[8]

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[1] *Pflüger's Archiv*, iii. 448.

[2] *Clinical Chem. of Urine*, p. 97.

[3] *Ber. d. deutsch. chem. Ges.* ix. 138.

[4] Senator, *Zeit. physiol. Chem.* iv. 1.

[5] *Centr. med. Wiss.* 1872, No. 1.

[6] *Maly's Jahresb.* 1874, p. 221.

[7] *Zeit. physiol. Chem.* ii. 273.

[8] G. Hoppe-Seyler, *Zeit. physiol. Chem.* xii. 1. F. Hoppe-Seyler, however, states that he has found great variations in the relative and actual amounts of both salts without assignable cause (*Physiol. Chem.* p. 846).

Otto[1] obtained a red pigment which he considered to be formed from the skatoxyl-sulphate as indigo is from the indoxyl-sulphate. Mester,[2] however, who fed a dog on skatole, found only traces of the skatoxyl salt in the urine,[3] but abundance of the skatoxyl-pigment. He gives certain reactions of this pigment, and considers it identical with those previously described under the names of urorubin, urorosein, uroerythrin, &c. In the urine it exists as a chromogen of unknown nature, perhaps a compound of skatoxyl with glycuronic acid, analogous to indoxylglycuronic acid. On adding mineral acids to the urine containing it, it became red or reddish violet, especially on warming. The pigment is probably an oxidation product of the chromogen.

The following table (adapted from Hoppe-Seyler) will be found convenient for the separation of some of these substances:—

<table>
<tr><td colspan="3">Urine is evaporated to one-third of its volume, and shaken with ether; this takes up pyrocatechin and hydroquinon</td></tr>
<tr><td colspan="2">A. The ethereal extract
Evaporate off the ether, and dissolve residue in water. Add lead acetate. This produces a precipitate. Filter this off</td><td rowspan="2">B. The residue after extraction with ether. Render acid with sulphuric acid; put it into retort and distil. In the distillate PHENOL will be found</td></tr>
<tr><td>Precipitate
Dissolve in water; pass a stream of sulphuretted hydrogen through it to precipitate lead. Filter off the lead sulphide; concentrate the filtrate, shake it with ether; evaporate the ether from the ethereal extract. Residue = PYROCATECHIN</td><td>Filtrate
Separate lead as before. Shake final filtrate with ether: evaporate ether from ethereal extract: the residue is HYDROCHINON</td></tr>
</table>

The following table gives in a concise way some important reactions of these substances:—

Substance sought	Test	Reaction	Remarks
Pyrocatechin .	This may be obtained from urine directly by evaporating to a syrup, extracting with alcohol, evaporating the alcohol from the extract, and taking up the residue with ether. Evaporate off the ether from the extract, and take up residue with water		It is this substance and hydrochinon which give to alkaline urine a dark colour at the surface (due to oxidation). It is present in abundance in 'carboluria'

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[1] *Pflüger's Archiv*, xxiii. 614.

[2] *Zeit. physiol. Chem.* xii. 180.

[3] Using G. Hoppe-Seyler's method of isolating these substances (*Zeit. physiol. Chem.* vii. 428).

Substance sought	Test	Reaction	Remarks
Pyrocatechin .	Add weak ferric chloride	It is coloured green, which passes into violet on adding acetic acid and ammonia	
	Render the solution alkaline	On exposure to the air it becomes yellow, then brown, or even black	
	Add lead acetate	It is precipitated	
Hydrochinon .	Will be found in the ethereal extract, made as in preceding table		
	A watery solution, with ammonia added	Is coloured brown	
	Sublimed	Yields an indigo-blue sublimate	
Oxy-acids . .	Obtained by shaking urine made acid with strong mineral acid with ether, evaporating off the ether from the extract, and taking up the residue with water. Add Millon's reagent	An intense red colour	
Phenol . . .	500 c.c. of urine are treated with excess of bromine water	A turbidity appears at first, passing on standing for several hours into a distinct yellow precipitate of tribromophenol	Cresol gives the reactions except with ferric chloride method of separat from urine is giv p. 743
	Distil urine acidulated with sulphuric acid	Phenol will be found in the distillate; add bromine water; a precipitate of tribromophenol appears	
	Warm the distillate with Millon's reagent	A cherry red colour	
	Add ferric chloride to the distillate	A deep violet colour	
	Decolourise urine with animal charcoal; dip a pine chip in hydrochloric acid containing a little potassium chlorate, and moisten it with the urine	It turns blue in sunlight	

CHAPTER XLI

THE PIGMENTS OF THE URINE

The pigments of the urine have been described under different names by different observers, and in the following account of them I shall follow MacMunn[1] very closely. It will also be convenient here to describe, not only the normal pigments, but also those occurring in disease.

NORMAL UROBILIN

This is the principal colouring matter of normal urine. It may be obtained from the urine by adding neutral and then basic lead acetate until there is no further precipitate. The precipitate consists of the chloride, sulphate, and urate of lead, and it carries down with it most of the pigment; it is filtered off; the filtrate is clear and almost colourless. The pigment is extracted from the precipitate by alcohol acidulated with sulphuric acid; the extract is filtered off from the remainder of the precipitate which is insoluble in this reagent. The extract has a deep yellowish colour; it is agitated with chloroform. This reagent dissolves out the pigment, which is obtained in an approximately pure condition by evaporating the chloroform from the chloroformic extract.

Normal urobilin thus obtained is amorphous, yellowish brown in colour, freely soluble in alcohol, chloroform, acids, acidulated water, and partly soluble in ether and benzene. An acid solution of it shows spectroscopically one absorption-band close to and enclosing the F line. If the solution be made neutral by alkalis, the band disappears. If the absorption spectrum of normal urobilin (fig. 97, spectrum 1, p. 748) be compared with that of choletelin (fig. 88, spectrum 2, p. 685), it will be found that the two are practically identical. It would, however, be premature to say that the two substances are identical, until spectroscopic analysis is supported by other analytical methods. When normal urobilin is separated out and dissolved in alcohol, and treated with zinc chloride and ammonia, the solution shows a green fluorescence, which, however, is not nearly so well marked as that obtained by treating a solution of hydrobilirubin in the same way. The spectroscopic appearances are also different. Spectrum 4 (fig. 88) gives the absorption-bands of hydrobilirubin after this treatment; the band at F becomes narrower, and shifts nearer to the *b* line, and there are two other bands near the C and D lines respectively. The spectrum of urobilin, treated with zinc chloride and ammonia, is very similar. The band at F shifts nearly to the *b* line, becoming somewhat narrower at the same time, and two new bands at the red end of the spectrum make their appearance. They are fainter and narrower than the similar bands just described in connection with hydrobilirubin, and they have a slightly different position.

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[1] *Clinical Chemistry of Urine*, pp. 104–112.

Origin of normal urobilin.—The theory formerly advanced as to the origin of normal urobilin was that bilirubin entering the intestine with the bile was acted upon by nascent hydrogen generated by putrefaction processes, and that a reduction product was formed, which Maly[1] considered was identical with one which he had prepared artificially from bilirubin by the action of sodium-amalgam, and which he called hydrobilirubin. The name stercobilin was given to the pigment of the fæces by Vaulair and Masius.[2] It was further supposed that the pigment of the fæces was in part absorbed from the alimentary canal, carried to the kidneys and there excreted. Hydrobilirubin, stercobilin, and urobilin were thus considered to be different names for the

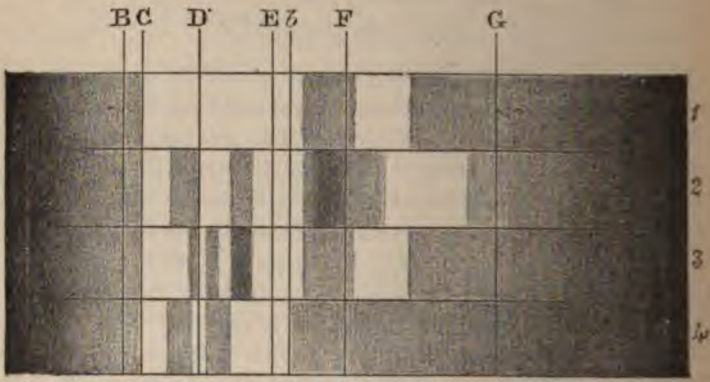

FIG. 97.—1. Absorption-spectrum of normal Urobilin; rectified spirit and sulphuric acid extract from the precipitate obtained by treating normal urine with neutral and basic lead acetate; 2. Absorption-spectrum of pathological urobilin; solution prepared in the same way; 3. Absorption-spectrum of urohæmatoporphyrin; solution prepared in the same way; 4. Indigo blue and indigo red from normal urine. The urine was boiled with an equal bulk of hydrochloric acid, and when cold agitated with chloroform. The same spectrum may be obtained by treating the urine by Jaffe's method and agitating with chloroform. The bluer the chloroform, the darker is the band before D; the nearer it approaches red the darker that after D. A third band at F may be also seen, due to urobilin (after MacMunn).

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[1] *Ann. Chem. Pharm.* clxi. 368; clxiii. 77.

[2] *Centralbl. med. Wiss.* 1871, No. 24.

same pigment; but recent spectroscopic research has shown differences between them (*see* also p. 697).

MacMunn is inclined to regard the formation of normal urobilin rather as the result of oxidation processes by means of the nascent oxygen in the intestine or elsewhere in the body than as due to reduction processes. This view is based chiefly on the fact that by the action of hydrogen peroxide on acid hæmatin, he is able to prepare an artificial product which shows the same spectroscopic appearances as normal urobilin.[1] Hoppe-Seyler[2] had previously prepared an artificial urobilin from hæmoglobin, and also from hæmatin, by the action of tin and hydrochloric acid. Whether stercobilin and urobilin are to be looked upon as products of reduction or oxidation must, therefore, still be regarded as unsettled. The most important point to notice, however, is that urobilin may originate either from bile-pigment or from blood-pigment.

We have seen that stercobilin and urobilin are different spectroscopically. The question next arises, are they different also in origin, or is urobilin simply stercobilin which has been somewhat changed in the processes of absorption and excretion? This question cannot be answered positively; there are, however, certain facts which seem to point to the conclusion that the processes that form the two pigments are, to a certain extent at any rate, independent of one another. These facts are as follows:—

(1) In animals with a biliary fistula, no bile enters the intestine; still the urine contains urobilin.

(2) In Copeman and Winston's[3] case of biliary fistula in a woman, in whom no bile entered the intestine, and whose fæces were uncoloured by stercobilin, the urine still contained urobilin.

(3) Some cases recorded by Mott (*see* p. 552) seem to locate the formation of normal urobilin in the liver. For when the destruction of red corpuscles is excessive in the portal circulation, the liver contains more iron than usual, and the iron-free residue of hæmoglobin appears in the urine as urobilin in abnormally large quantities.

The quantity of urobilin in the urine seems to be increased by oxidation, for instance, by a little dilute potassium permanganate, by hydrochloric or nitric acid, or by the occurrence of the acid fermentation. It is supposed that the greater part of the urobilin present is in the form of a colourless chromogen, which, on oxidation, is converted into the pigment (Hoppe-Seyler, MacMunn).

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[1] *Journ. of Physiol.* x. 112.

[2] *Pflüger's Archiv*, x. 208.

[3] *Journ. of Physiol.* x. 21.

PATHOLOGICAL UROBILIN

This substance is sometimes termed febrile urobilin, as it appears in certain febrile conditions.

It is prepared from urine by the same method as that already described for normal urobilin, and is soluble in the same reagents.

Its spectrum is shown in fig. 97, spectrum 2. The band at F is darker and wider than the corresponding band of normal urobilin; there are in addition two other bands in acid solutions, one between D and E and the other just before D.

On treatment with zinc chloride and ammonia a deep green fluorescence is developed, as with stercobilin and hydrobilirubin, and the spectrum then seen is a three-banded one practically identical with that obtained by treating normal urobilin in the same way, and also like that obtained by the similar treatment of hydrobilirubin. Stercobilin, on the other hand, after this treatment gives a four-banded spectrum.

Pathological urobilin can be artificially prepared from artificial normal urobilin by the action of reducing agents. We may therefore infer that pathological urobilin is a less oxidised stage of the same material which under normal circumstances passes into normal urobilin.

Pathological urobilin, like normal urobilin, does not necessarily arise from bile-pigment. It may arise from the blood-pigment. After extensive extravasations of blood into the tissues, or into the peritoneum, the urine becomes dark, like jaundiced urine, but the pigment is found to be pathological urobilin.[1]

UROHÆMATOPORPHYRIN

This pigment has been found by MacMunn and subsequently by le Nobel[2] in certain diseased conditions, viz. Addison's disease, acute rheumatism, cirrhosis of the liver, pneumonia, pericarditis, peritonitis, measles, meningitis, typhoid fever, and Hodgkin's disease. MacMunn considers it probably closely related to Baumstark's[3] ururubrohæmatin and urofuscohæmatin. Like urobilin, it may exist in the urine partly in the form of a chromogen (named urobilinoidin by le Nobel), which on oxidation is transformed into the pigment.

It can be prepared from the urines that contain it by the same method as that already described for urobilin. It can be prepared artificially from hæmatin (not from bile-pigments) by the action of zinc and sulphuric acid, sodium-amalgam, and other reducing agents.

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[1] Cases of this condition which occurred in Univ. Coll. Hosp. under Dr. Ringer's care are described by MacMunn, *Journ. Physiol.* x. 83.

[2] *Pflüger's Archiv*, vol. xl. 1887.

[3] *Ibid.* ix. 568.

In acid solutions the spectrum is characteristic (fig. 97, spectrum 3); the bands are four in number: a narrow one before and touching D, another darker between D and E, a feeble shading between these two, and, lastly, a band at F, practically identical with that of normal urobilin. If the pigment is dissolved in alcohol, and ammonia added, a five-banded spectrum like that of neutral hæmatoporphyrin is obtained. Treated with zinc chloride and ammonia, a faint green fluorescence appears; the band at F becomes a little narrower and shifts a little towards the red end of the spectrum, and there are two other bands between D and E. This pigment thus resembles the pigments we have previously discussed, but shows certain differences from all of them.

More recently MacMunn (*Proc. Physiol. Soc.* 1890, p. xiii.) found in three specimens of morbid urine a pigment probably intermediate between urohæmatoporphyrin and hæmatoporphyrin.[1] These urines were of a deep Burgundy red colour, contained no proteid, and, on the addition of a drop of sulphuric acid, showed the spectrum of acid hæmatoporphyrin (fig. 59, spectrum 10, p. 277).

The table on the next page collects together the chief distinguishing characters of these various but still closely allied pigments.

OTHER URINARY PIGMENTS

Indigo.—*See* p. 744.

Skatole pigment.—*See* p. 745.

Urorubin, uroroseïn, purpurin.—Probably identical with the skatole-pigment (Mester).

Uroerythrin.—This is the pigment which colours deposits of urates a brick-red tint. Mester considers that this also is identical with skatole-pigment. MacMunn, however, states that it gives certain characteristic reactions. It may be extracted from the urates by boiling alcohol. This solution gives two ill-defined bands before F. In the solid state it becomes green with caustic soda or potash. Its origin and its relation to urobilin are unknown.

Urochrome.—This was the name given by Thudichum[2] to what he considered to be the chief urinary pigment. It is possibly impure urobilin altered by the method of preparation. It may be prepared as follows:—Precipitate about 50 c.c. of urine with lead acetate and a drop of ammonia. Filter. The filtrate is colourless. Scrape the precipitate into a capsule, mix it with a few drops of sulphuric acid, and add to the pasty mass a little alcohol. Filter. A yellow alcoholic solution of urochrome comes through. Boil this with excess of sulphuric acid, and dilute the acid liquid with water. Black flocculi are formed: these do not consist of carbon produced by charring, as they are readily soluble in ammonia, from which it can be again precipitated by sulphuric acid. The name *uromelanin* was given to this black pigment. Thudichum described another derivative of urochrome, which he named *uropittin* ($C_9H_{10}N_2O_3$). These experiments have now a merely historical interest.

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[1] *See* also Ranking and Pardington, *Lancet*, ii. 1890, p. 607.

[2] *Brit. Med. Journ.* November 1864, p. 509.

Pigment	*Hydrobilirubin*	*Stercobilin*	*Normal urobilin*	*Pathological urobilin*	*Urohæmato-porphyrin*
Definition	A pigment artificially produced from bilirubin by reduction with sodium-amalgam	The pigment of the fæces	The pigment of normal urine	Pigments occurring in the urine in certain diseases, chiefly of a febrile character	
Origin in the body		A reduction-product from bilirubin. (An oxidation-product from bilirubin, and partly from the hæmoglobin of the food MacMunn)	A reduction-product from bile-pigment or blood-pigment. (Oxidation-product – MacMunn)	A less highly oxidised product from bile- or blood-pigment (MacMunn)	A reduction-product from blood-pigment (MacMunn)
			These pigments all exist in the urine mostly as chromogens, which by oxidation are converted into the pigments		
Spectroscopic appearances	Two bands, one at D, the other between *b* and F (fig. 88, spectrum 3)	Two bands, one at D, the other between *b* and F	One band at F (like choletelin) (fig. 97, spectrum 1)	Three bands, one just before D, one between D and E, the third dark and wide at F (*ibid.* spectrum 2)	Four bands, one just before D, two between D and E, the fourth at F (*ibid.* spectrum 5)
On treatment with zinc chloride and ammonia	Well-marked green fluorescence. *Spectrum*: three bands, one after C, one at D, the third between *b* and F (fig. 88, spectrum 4)	Well-marked green fluorescence. *Spectrum*: four bands, one after C, one at D, one between D and E, the fourth between *b* and F	Fairly well-marked green fluorescence. *Spectrum*: three bands, like those of hydrobilirubin	Well-marked green fluorescence. *Spectrum*: three bands, like those of hydrobilirubin	Faint green fluorescence. *Spectrum*: three bands, two between D and E, the third between *b* and F

Melanin.—This substance must not be confused with the artificial product named uromelanin just described. Melanin, or a chromogen called melanogen, is converted into melanin by oxidation, occurs in the urine in some cases of melanotic sarcoma (*see* p. 499), and the term *melanuria* may be employed to denote this condition. The melanin probably contains iron. v. Jaksch[1] has described two cases of this condition. He finds that the urine becomes dark brown or black on addition of a dilute solution of ferric chloride. In urine containing melanin or its precursor melanogen, Prussian blue is formed on adding a nitroprusside, aqueous potash, and an acid. This reaction, however, does not seem to depend on the presence of melanin, as it is not given by that substance when separated from the urine, but apparently by some other, at present unknown, substance, which is present in traces in normal urine, and is increased in cases of melanuria, and also in those conditions where excess of indigo occurs in the urine.

Humous substances.—When an apple is cut open, it becomes dark on exposure e air: this is a familiar instance of a widespread occurrence in the vegetable l, the formation of a humous substance. These humous substances are l to carbohydrates, and also to aromatic bodies, as on fusing with potash yield pyrocatechin, protocatechuic acid, as well as volatile fatty acids. appear to be nitrogenous, and such a one is formed, according to nszky,[1] from urea and the normal carbohydrates of the urine on heating a mineral acid. The dark colour of herbivorous urine, and also that in luria, is stated by Udranszky to be due to similar humous pigments, and ding to him the pyrocatechin obtainable from such urines is due to the nposition of the humous substances they contain. The experiments, how- do not appear to me to fully bear out these statements, and until fuller is thrown on the subject, Udranszky's conclusions must be accepted with on.

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[1] *Zeit. physiol. Chem.* xiii. 385. v. Jaksch points out that a large quantity of melanin may occur in the urine in wasting diseases, and it may be absent in cases of melanotic sarcoma. *Clinical Diagnosis*, p. 249.

[1] *Zeit. physiol. Chem.* ii. 587; xii. 33.

CHAPTER XLII

OTHER ORGANIC CONSTITUENTS OF THE URINE

A NUMBER of organic constituents, in addition to those already described, may occur in small quantities in the urine. We may divide these into the following groups :—

(1) Non-nitrogenous acids : oxalic, succinic, and lactic acids.
(2) Fatty acids.
(3) Glycero-phosphoric acid.
(4) Carbohydrates : dextrose, animal gum.
(5) Ferments : especially pepsin.
(6) Mucin.
(7) Cynurenic and urocanic acids.

Oxalic acid ($C_2H_2O_4$).—The free acid never occurs in the urine, but it is united with calcium to form an oxalate, which, under ordinary circumstances, is held in solution in the urine by the acid phosphate of sodium. Schultzen[1] found that 0·1 gramme (1·5 grain) was excreted daily by men. Neubauer in some cases found it wholly absent. It is much more abundant in the urine of horses and pigs.

It occurs in excess in the urine after the ingestion of rhubarb and cabbage, the former of which contains an especially large amount of the acid.

It is increased in a condition called 'oxaluria,' in which the most prominent subjective symptom is nervous depression. Oxaluria occurs in a variety of ailments ; an increased secretion of uric acid is generally accompanied with an increase of oxalic acid. It also occurs in excess in certain cases of catarrh of the urinary passages.

When present in excess it is in the form of a precipitate of crystals of calcium oxalate. Such crystals are frequently found in the 'lateritious deposit' of febrile urine. Such crystals form, as a rule, after the urine has stood a few hours, especially if it contains excess of mucus, or spermatozoa, as in spermatorrhœa. This deposition of crystals is probably the result of an acid fermentation.

Crystals of calcium oxalate ($C_2CaO_4 + 2H_2O$) are distinguished by their form, quadratic octahedra with a short principal axis ; these are often termed 'envelope crystals' (fig. 98). Occasionally dumb-bell forms are seen. The crystals are further characterised by their extreme insolubility; they are insoluble in ammonia, in acetic acid, and soluble with difficulty in dilute hydrochloric acid.

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[1] *Arch. f. Anat. u. Physiol.* 1868, p. 719.

On account of the insolubility of calcium oxalate, oxalic acid is generally estimated as the calcium salt. The origin of oxalic acid in the body is uncertain. Frerichs and Wohler found that dogs fed on uric acid had an increase of calcium oxalate in their urine. The close relationship between uric acid and oxalic acid appears to be undoubted, as may be seen by consulting the account already given of uric acid (p. 729).

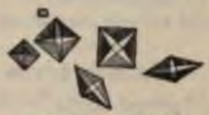

FIG. 98.—Crystals of Calcium Oxalate.

Calculi consisting of calcium oxalate (mulberry calculi) are exceedingly hard and insoluble. Stones consisting of a mixture of uric acid and calcium oxalate are fairly common.

Succinic acid ($C_4H_6O_4$), the third term of the series of acids of which oxalic acid is the first, has been occasionally found in the urine (Meissner[1]), especially after the ingestion of asparagus (Hilger[2]). Salkowski,[3] v. Longo[4] (after ingestion both of asparagus and asparagine), and Baumann[5] (after ingestion of sodium succinate) failed to find it in the urine.

Lactic acid ($C_3H_6O_3$).—This probably does not occur in normal urine. It has been found in the urine combined with bases after great muscular activity;[6] according to Colasanti and Moscatelli[7] the form of lactic acid which then occurs is sarcolactic acid. It has also been found in cases of trichinosis,[8] acute yellow atrophy of the liver,[9] liver cirrhosis,[10] diabetes,[11] phosphorus poisoning,[12] rickets,[13] leucocythæmia,[14] osteomalacia,[15] and in animals after extirpation of the liver[16] (*see* p. 735).

Fatty acids.—These occur in normal urine in mere traces (0·008 gramme daily). They consist of formic, acetic, butyric, and propionic acids, and the amount in the day's urine can be increased by treating the urine with oxidising agents to 0·9 to 1·5 gramme (v. Jaksch[17]).

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[1] Meissner and Shepard, *Untersuchungen ü. d. Entstehen der Hippursäure*, Hanover, 1866.

[2] *Liebig's Ann.* clxxi. 208.

[3] *Pflüger's Archiv*, iv. 95.

[4] *Zeit. physiol. Chem.* i. 213.

[5] *Ibid.* p. 215.

[6] Spiro, *Ibid.* p. 117.

[7] *Gazz. Ital.* xvii. 548. The occurrence of lactic acid in the urine (except in frogs) after muscular work is denied by Marcuse, *Bied. Centralbl.* 1887, p. 92.

[8] Simon and Wibel, *Ber. d. deutsch. chem. Ges.* 1871, p. 139.

[9] Schultzen and Riess, *Ann. des Charité Krank.* xv. 1.

[10] Bunge, *Physiol. Chem.* trans. by Wooldridge, p. 345.

[11] Bouchardat, *Maly's Jahresb.* 1876, p. 155.

[12] Schultzen and Riess, *loc. cit.*

[13] Gorup-Besanez, *Lehrbuch*, 1878, p. 606.

[14] Körner and Jacubasch, *Arch. f. path. Anat.* xliii. 196.

[15] Moers and Myk, *Zeit. anal. Chem.* 1869, p. 520; *Arch. f. klin. Med.* v. 486.

[16] Minkowski (in geese), *Arch. exp. Path. und Pharmak.* xxi. 41; Marcuse (in frogs), *Pflüger's Archiv*, xxxix. 425; Nebelthau (in frogs), *Zeit. Biol.* xxv. 123. The acid appears to be sarcolactic (Nebelthau).

[17] *Zeit. physiol. Chem.* x. 536.

The amount of fatty acids also increases during the occurrence of the ammoniacal fermentation of urea (Salkowski).[1]

The amount of fatty acids in the urine is increased in certain febrile conditions to 0·06 gramme, and in certain liver diseases to 0·6 to 1 gramme *per diem*. This condition is called *lipaciduria* by v. Jaksch. The acids are apparently free in the urine.

Glycero-phosphoric acid ($C_3H_9PO_6$) occurs in normal urine[2] to the extent of 15 milligrammes per litre. It is increased in nervous diseases (Lepine) and after chloroform narcosis (Zuelzer).

Carbohydrates.—*Dextrose.*—The occurrence of abundant quantities of grape sugar in the urine is one of the most prominent symptoms of the disease called diabetes. The question, however, we have now to consider is, Does sugar occur in normal urine? The answer has been sought by many observers, a large number of whom state that sugar is, and a nearly equally large number of whom state that it is not present.[3] The conclusion one would draw from a list of references such as is given below is, that if sugar is present at all, it occurs in very small quantities. The difficulty of the investigation is increased by the fact that urine contains several substances that reduce alkaline solutions of cupric hydrate; these are uric acid, hippuric acid, pyrocatechin, glycuronic acid, and creatinine. None of these, however, undergo the alcoholic fermentation on the addition of yeast, and this does take place with the reducing substance of normal urine (Abeles). Some of the older opponents of the view that urine contains sugar said that, even if sugar is formed, it is the result of the decomposition of indican; this, of course, was when physiologists held the idea that the indican of urine, like that of plants, was a glucoside. We have already seen (pp. 73 and 743) that the so-called indican of urine is not a glucoside. The balance of evidence appears to me to be clearly in favour of the existence of a small quantity of dextrose in normal urine. The most recent work at the subject is that of Wedenski. He shook up a large quantity of normal urine with benzoic chloride; by this treatment insoluble benzoyl compounds of carbohydrates, if present, separate out.

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[1] *Zeit. Physiol. Chem.* xiii. 264. Salkowski considers that during the fermentation they originate from the carbohydrates of the urine. *See* also Tanigati, *ibid.* xiv. 471.

[2] Klüpfel and Fehling, Sotnichewski, *Zeit. physiol. Chem.* iv. 214.

[3] For most of the following references I am indebted to Hoppe-Seyler's *Physiol. Chem.* p. 828. Those who state sugar is present are: Brücke (*Wien. Akad. Sitzungsber.* xii. 346), Bence Jones (*Chem. Soc. Quart. Journ.* xiv. 22), Tuchen (*Virchow's Archiv*, xxv. 26), Ivanoff (*Diss.* Dorpat, 1861), Meissner and Babo (*Zeit. rat. Med.* (3), ii.), Pavy (*Guy's Hosp. Rep.* xxi. 413), Abeles (*Centralbl. med. Wiss.* 1879, Nos. 3, 12, and 22), Udranszky (*Zeit. physiol. Chem.* ii. 537; xii. 33), Wedenski (*Ibid.* xiii. 122), Salkowski (*Ibid.* xiii. 264), Hagemann (*Pflüger's Archiv*, xliii. 501). Those who state that sugar is absent are: Friedländer (*Arch. d. Heilk.* vi. 97), Maly (*Wien. Akad. Sitzungsb.* lxiii. 3), Seegen (*Ibid.* lxiv. 2), Külz (*Pflüger's Archiv*, xiii. 269).

Such a precipitation does occur. Elementary analysis showed the probable presence of two carbohydrates; these were separated by treatment with soda; part remains undissolved, and gives the reactions of dextrose; the part that dissolves gives the characters of animal gum.

Animal gum.—This carbohydrate radicle of mucin (*see* p. 480) was originally found in the urine by Landwehr.[1]

Milk sugar.—This often occurs, but in small and variable quantities, in the urine of nursing mothers (Blot,[2] de Sinetz,[3] Hofmeister,[4] Kaltenbach[5]). Hofmeister precipitated urine with lead acetate and ammonia, filtered, decomposed the filtrate with sulphuretted hydrogen to get rid of lead, filtered, shook the filtrate with silver oxide, filtered, decomposed the filtrate with sulphuretted hydrogen to get rid of silver, filtered; to the final filtrate barium carbonate was added, and the mixture evaporated to dryness. Alcohol removed milk sugar from the residue, and characteristic crystals of it were obtained by evaporating off the alcohol. Kaltenbach further showed that this substance was milk sugar, as he obtained galactose and mucic acid from it.

Inosite.—Small quantities of inosite have been found in normal urine by Cloetta,[6] Gallois,[7] Strauss,[8] Külz[9]; in the urine of cases of Bright's disease by Cloetta, and in diabetic urine by Mosler and Schwanert.[10] Dahnhardt[11] obtained 0·1 gramme of inosite from 8 kilos. of oxen's urine. Feeding with inosite does not increase the amount in the urine (Külz).

It may be detected in the urine as follows[12]:—Several litres of urine feebly acidified are completely precipitated with lead acetate and filtered. The filtrate is warmed and completely precipitated with basic lead acetate. After standing forty-eight hours the precipitate is collected, washed, suspended in water, and treated with a stream of sulphuretted hydrogen; the lead sulphide is filtered off. Uric acid separates from the filtrate after some hours; this also is filtered off. The solution is then evaporated to a syrup on the water-bath, and absolute alcohol added. The precipitate is dissolved in hot water, and three or four times the volume of 90 per cent. alcohol added. Ether is cautiously added till a permanent cloud appears; the inosite crystallises out, and may be collected; it will then give its characteristic tests (p. 101).

Ferments.—*Pepsin.*—Several observers (Brücke, Sahli,[13] &c.) have found pepsin in the urine. The following is an abstract of Leo's[14] work on the subject. Small pieces of fibrin soaked in the urine absorb the pepsin therefrom; on removing them to 0·1 per cent. hydrochloric acid they are rapidly digested. Control experiments with fibrin not previously soaked in urine gave negative results. Morning urine is richest in pepsin.

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[1] *Centralbl. med. Wiss.* 1885, p. 369.

[2] *Compt. rend.* xliii. 676.

[3] *Gaz. méd. Paris*, 1873, p. 573.

[4] *Zeit. physiol. Chem.* i. 101.

[5] *Ibid* ii. 360.

[6] *Ann. Chem. Pharm.* xcix. 289.

[7] *Thèse*, Paris, 1864.

[8] *Diss.* Tübingen, 1870.

[9] *Centralbl. med. Wiss.* 1875, p. 933.

[10] *Arch. pathol. Anat.* xliii. 229.

[11] *Arbeit aus d. Kieler physiol. Inst.* 1868, p. 157.

[12] Salkowski and Leube, *Lehre vom Harn.*

[13] *Pflüger's Archiv*, xxxvi. 209.

[14] *Ibid.* xxxvii. 223; xxxix. 246.

Neumeister[1] found pepsin in the urine of the dog, but not in that of the rabbit. Neumeister and Stadelmann[2] both showed that the ferment in the urine is true pepsin; it forms peptone and all the intermediate proteoses from fibrin just as pepsin does.

Trypsin.—Except Sahli, all observers agree that trypsin is absent from the urine. Sahli's results were probably due to the non-prevention of putrefaction in his experiments. Pieces of fibrin soaked in urine, according to Leo's method, are not digested in 1 per cent. sodium carbonate solution, thymol being added to prevent putrefaction. As trypsin is not found in the blood or tissues, Leo concludes that it is entirely destroyed in the alimentary canal; while the pepsin is not wholly destroyed there, but is partly absorbed, and passes into the blood, tissues, and urine. By making extracts of the different parts of the intestine, Leo draws the conclusion that pepsin disappears in the second third and trypsin in the lower third of the small intestine.

Diastatic ferment.—Holovotschiner[3] states he has obtained small quantities of ptyalin or a similar diastatic ferment from urine.

Rennet.—Holovotschiner and Helwes[4] both obtained from urine traces of a ferment which curdles milk.

Mucin.—This is the chief constituent of the mucus derived from the urinary passages. It occurs in normal urine in small quantities; in catarrhal diseases of the urinary passages it is increased. It is slightly soluble in neutral and alkaline urines, and may be precipitated therefrom by acetic acid (insoluble in excess) or by alcohol; it is not precipitated by boiling, and so may be distinguished from albumin. It is probably the source of the animal gum found by Landwehr in the urine.

Cynurenic and urocanic acids.—These are two peculiar acids, the characters of which are described on p. 91, and which hitherto have been found only in the urine of dogs. The former may be precipitated in crystals from urine by nitric acid. They are found in the urine of starving dogs, and so must be products of metabolism, and not the result of putrefaction in the intestines.[5]

Kryptophanic acid ($C_5H_9NO_5$) was described by Thudichum as a normal constituent of urine, but has not been found by anyone else.

Nephrozymase is a substance precipitated by alcohol from urine by Béchamp. According to him, it is proteid in nature. Normal urine, however, is absolutely free from proteids.

Urethan (ethyl carbamate) is found in small quantities in the alcoholic extract of normal urine. It is, however, an artificial product of the action of alcohol on urea (Jaffe and Cohn).[6]

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[1] *Zeit. Biol.* xxiv. 272.

[2] *Ibid.* xxv. 208.

[3] *Chem. Centralbl.* 1886, p. 327.

[4] *Pflüger's Archiv*, xliii. 384.

[5] Baumann, *Zeit. physiol. Chem.* x. 123.

[6] *Ibid.* xiv. 395.

CHAPTER XLIII

THE INORGANIC CONSTITUENTS OF URINE

The inorganic constituents of the urine are chiefly chlorides, carbonates, sulphates, and phosphates; the metals with which these are in combination are sodium, potassium, ammonium, calcium, and magnesium. Small quantities of fluorine, silicic acid, and iron also occur; and the free gases present are carbonic acid and nitrogen, with traces of oxygen. The total amount of salts varies from 9 to 25 grammes daily. The inorganic salts of the urine are derived from two sources: first, from the food; the salts pass into the blood, and then are excreted by the kidneys; secondly, as a result of metabolic processes; this is especially the case with the phosphates, and more particularly still with the sulphates. The salts of the blood and those of the urine are much the same, with the important exception that, whereas the blood contains only traces of sulphates, the urine contains abundance of these salts; the sulphates are derived from the changes that occur in the proteids of the body; the nitrogen of the proteids is excreted as urea and uric acid; the sulphur is oxidised to form sulphuric acid, which passes into the urine chiefly combined with metallic bases, but to a small extent also in ethereal combinations with organic radicles to form the ethereal sulphates we have already considered (p. 740). The excretion of sulphates, moreover, runs parallel to that of urea. The tests for the chief salts are given on p. 717. Their estimation is described in Chapter XLV.

THE CHLORIDES

The principal chloride in the urine is that of sodium. Small quantities of potassium chloride and traces of calcium and magnesium chloride are also present. Sodium chloride is, in fact, the most abundant salt in the urine, as it is in the blood and in most other fluids of the body. Vogel gives the daily amount of chlorine excreted as 6 to 8 grammes, which would correspond to 10 to 13 grammes of sodium chloride.

The ingestion of sodium chloride in the food is followed by its appearance in the urine, some on the same day, some on the next day

(Dehn[1]); some, however, is decomposed to form the hydrochloric acid of the gastric juice. The sodium chloride, however, does not merely pass through the body without making its effect felt; it stimulates metabolism and secretion, as has already been pointed out (p. 61).

The urine is richest in sodium chloride after a meal; poorest at night time.[2] Drinking large quantities of water or beer increases it; a rich secretion of gastric juice causes a temporary decrease in the chlorides of the urine.[3] Certain chlorine compounds other than chlorides causes an increase of the urinary chlorides: chloroform narcosis,[4] and the administration of ethyl trichloracetate act in this way; whereas certain other chlorine compounds (chloral,[5] carbon tetrachloride, methyl chloride, &c.) do not have this effect (Kast[6]).

The quantity of chlorides excreted varies greatly in disease:—It is diminished in most febrile diseases; the cause of this is unknown, but is, perhaps, partially due to diminished intake of the salt, or in some cases to diarrhœa, by means of which a certain quantity of salt passes out per rectum (*see* p. 699). The decrease is especially marked in pneumonia, pleurisy, and typhoid fever, and runs parallel to the height of the fever. In pneumonia the chlorides may entirely disappear from the urine, their reappearance being one of the signs of improvement. It is diminished in cholera, chorea, and pemphigus. It is increased in diabetes, polyuria, and some forms of Bright's disease, where a large amount of urine is excreted.

The relation of sodium and potassium salts in some of these conditions has been investigated by E. Salkowski.[7] In health the ratio is variable, depending to some extent on diet. In febrile conditions in which the total chlorides are diminished the excretion of the potassium salt rises above the average. Zeelzer[8] states that the same occurs in conditions of excitement.

THE SULPHATES

The sulphates in the urine are of two kinds, ordinary sulphates of potassium and sodium (pre-formed sulphuric acid), and ethereal sulphates (combined sulphuric acid). They are derived in small measure from the food (administration of sodium or magnesium sulphate increasing the quantity of sulphates in the urine),[9] but chiefly from the metabolism of proteids in the tissues. The ethereal sulphates are the result of putrefaction of proteids in the intestines or elsewhere, as in a putrid abscess. The sulphates of the urine may vary in amount from 1·5 to 3 grammes daily (Furbringer,[10] Neubauer[11]). The administration of free sulphuric acid to dogs increases the urinary sulphates[12]; in rabbits this is not the case.[13]

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[1] *Pflüger's Archiv*, xiii. 353.

[2] A. Hegar, *Ueber d. Ausscheidung d. Chlor durch den Harn*, Giessen, 1852.

[3] *Chem. Centralbl.* 1887, p. 1561.

[4] Zeller, *Zeit. physiol. Chem.* viii. 70; Kast, *Ibid.* ii. 277.

[5] Chloral passes into the urine as urochloralic acid (v. Mering).

[6] *Loc. cit.*

[7] *Pflüger's Archiv*, iii. 351.

[8] *Centralbl. med. Wiss.* 1877, Nos. 44 and 45.

[9] Sick, *Diss.* Tübingen, 1859.

[10] *Arch. path. Anat.* lxxiii. 39.

[11] Neubauer and Vogel's *Text-book*.

[12] Frey and Gähtgens, *Centr. med. Wiss.* 1872, No. 53; Kurtz, *Diss.* Dorpat, 1874.

[13] Salkowski, *Arch. path. Anat.* lviii. 1.

The variations of the amount of urinary sulphates in disease can be almost guessed after the statement has been made that their amount runs parallel to that of the urea excreted. In conditions where metabolism is increased (fever, diabetes) the sulphates are increased; in conditions where metabolism is diminished (convalescence from fever, most chronic affections) the sulphates are diminished. Bence Jones states that an increase occurs in various forms of delirium; also in acute inflammatory diseases of the brain and spinal cord.

THE CARBONATES

Carbonate and bicarbonate of sodium, calcium, magnesium, and ammonium are generally present in fresh, alkaline urine. They arise in the organism from carbonates of the food, or from lactic, malic, tartaric, succinic, and other vegetable acids in the food. They are thus most abundant in the urine of herbivora and vegetarians, whose urine, we have already seen, is thus rendered alkaline. Urine containing carbonates is either cloudy when passed or, like saliva (*see* p. 622), soon becomes so on standing; the deposit, if allowed to settle, will, on examination, be found to consist of calcium carbonate and also phosphates.

THE PHOSPHATES

Phosphoric acid in normal urine occurs in the form of two classes of phosphates :—

(1) Alkaline phosphates. Phosphates of sodium are the most abundant; those of potassium scanty.

(2) Earthy phosphates. Phosphates of calcium are the most abundant; those of magnesium scanty.

The composition of the phosphates in urine is liable to variation. In acid urine, the acid salts are generally present, and give the urine an acid reaction. These are chiefly sodium dihydrogen phosphate (NaH_2PO_4) and calcium dihydrogen phosphate [$Ca(H_2PO_4)_2$]. In neutral urine, in addition to these, phosphates with formulæ Na_2HPO_4 (disodium hydrogen phosphate), $CaHPO_4$ (calcium hydrogen phosphate), and $MgHPO_4$ (magnesium hydrogen phosphate) are also found. In alkaline urine there may be in addition to, or instead of some of, the above the normal phosphates of sodium, calcium magnesium [Na_3PO_4, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$]. In addition to these, phosphoric acid may be united to the bases ammonia, urea, and creatinine.

The earthy phosphates are precipitated by rendering the urine alkaline by ammonia, potash or soda, or in the ammoniacal fermentation that occurs in decomposing urine. The alkaline phosphates remain in solution after the earthy phosphates have been precipitated in this way. The phosphates found most frequently in the white creamy precipitate that occurs in decomposing urine are (1) the triple phosphate (ammonio-magnesium phosphate, $NH_4MgPO_4 + 6\,H_2O$), which crystallises in triangular prisms, or so-called 'coffin-lid crystals' (fig. 99), and occasionally in feathery stellate crystals; (2) calcic phosphate, often called 'stellar phosphate,' which crystallises in star-like clusters of prisms.

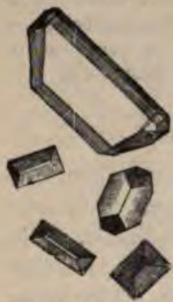

FIG. 99.—Ammonio-Magnesium or Triple Phosphate.

In acid urine a crystalline calcium phosphate occasionally separates out; it may also be obtained by adding calcium chloride to urine, or, after the internal administration of lime-water, or potassium carbonate. It has the composition $CaHPO_4 + 2H_2O$ (Hill Hassal,[1] Stein[2]).

Normal urine gives no precipitate when it is boiled. Neutral, alkaline, and, occasionally, faintly acid urine give a precipitate of calcium phosphate when boiled; this precipitate is amorphous, and is liable to be mistaken for albumin; it may be distinguished readily from albumin, as it is soluble in a few drops of acetic acid, whereas coagulated proteid does not dissolve. Salkowski[3] showed that the precipitated phosphates often redissolve when the urine cools.

There have been various explanations advanced to explain the precipitation of phosphates by heat. They all, however, may be summed up by saying that the phenomenon is the result of unstable equilibrium among certain phosphates, the balance of solubility being easily disturbed by changes of temperature and reaction, and possibly modified by the kind and amount of other salts in solution (W. G. Smith,[4] Stokvis[5]).

The precipitation was believed by some to be due to evolution of carbonic acid.

Salkowski attributes it to the decomposition of a compound of calcium an sodium phosphate.

Reynolds suggests that the change produced by heat may be represented as follows:—

$$2Ca_2H_2(PO_4)_2 + CaH_4(PO_4)_2 = Ca_3(PO_4)_2 \text{ (insoluble)} + 2CaH_4(PO_4)_2 \text{ (soluble)}.$$

A. Ott[6] speaks as follows on the subject. Erlenmeyer[7] has shown that acid calcium phosphate is soluble in 700 parts of water. But the urine is able to hold more than this in solution, in virtue of the presence of other salts. Similarly the normal phosphate is more soluble in urine than in water, such salts as potassium phosphate and sodium chloride aiding its solution. By heating an aqueous solution of the two phosphates the acid phosphate is changed into the normal phosphate, and is precipitated, phosphoric acid passing into solution. But in normal urine no such precipitation, or only a slight one, occurs, because of the presence of the other salts just alluded to. If, however, the normal relation between these salts be upset, then we get precipitation of the normal calcium phosphate.

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[1] *Proc. Roy. Soc.* x. 281.

[2] *Liebig's Annalen*, clxxxvii. 90.

[3] *Zeit. physiol. Chem.* vii. 119.

[4] *Dublin Journ. Med. Sci.* July 1883.

[5] *Chem. Centralbl.* 1884, p. 42.

[6] *Zeit. physiol. Chem.* x. 167.

[7] *Ber. d. deutsch. chem. Ges.* ix. p. 1839.

Origin of, and variations in, the urinary phosphates.—The phosphoric acid in the urine is partly derived from the food, and is partly a decomposition product of lecithin and nuclein. The amount of the acid in the twenty-four hours' urine varies from 2·5 to 3·5 grammes, of which the earthy phosphates constitute about half (1 to 1·5 gramme).

The excretion of phosphoric acid varies in amount with the food taken: after the midday meal, especially if it consists of meat, it rises, reaching its maximum in the evening; it falls during the night, reaching its minimum at midday. The average of Ott's analyses shows that the ratio of P_2O_5 combined as normal phosphate to that combined as acid phosphate was as follows:—Evening urine (2 to 10 P.M.), 91 : 100; night urine (10 P.M. to 8 A.M.), 58 : 100; morning urine (8 A.M. to 2 P.M.), 69 : 100.

An interesting point in connection with this subject is whether activity of the nervous system produces an increased output of phosphoric acid from increased metabolism of lecithin. Mendel[1] found it diminished in chronic brain diseases. Vanni and Pous,[2] with certain reservations, came to the same conclusion. Mairet[3] concludes that brain work increases the excretion of alkaline phosphates. The question, however, appears to me to be an especially difficult one to investigate; the quantity that arises from decomposition of brain substance must under any circumstances be small, and such small differences are particularly hard to recognise when one remembers, as Mairet himself points out, that effective brain work is difficult on an insufficient diet. The increase noted may just as probably be due to the food taken to sustain mental activity as to the mental activity itself.

Several observers have found that muscular work increases the output of phosphoric acid (Mosler, [4]Lehmann[5]), while others have found that it does not (Pettenkofer and Voit,[6] Byassan,[7] North[8]).

In various pathological conditions the output of phosphoric acid varies: it is diminished in gout,[9] in most acute diseases, probably because only a small amount of food is taken (Vogel), in kidney disease,[10] in the intervals of intermittent fever,[11] after large doses of chalk, ether, and alcohol, and during pregnancy (owing to the formation of fœtal bones). It is increased after copious draughts of water, after sleep produced by potassium bromide or chloral hydrate (Mendel[12]), in inflammation of the brain, in chorea, acute atrophy of the liver, phthisis, and leucocythæmia.

An increase of phosphates in the urine is termed *phosphaturia*. A deposit of earthy phosphates may be due to disturbance of the unstable equilibrium of the

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[1] *Arch. f. Psychiatrie*, vol. iii. 1872, p. 636.
[2] *Chem. Centralbl.* 1887, p. 1526.
[3] *Compt. rend.* xcix. 282.
[4] *Beiträge zur Kenntniss der Urinabsonderung*, Giessen, 1853.
[5] *Arch. f. Anat. u. Physiol.* 1871, p. 14.
[6] *Zeit. Biol.* ii. 459.
[7] *Essai*, Paris, 1868.
[8] *Proc. Roy. Soc.* xxxix. 443 (*see* also p. 437 of this book).
[9] Stokvis, *Centr. med. Wiss.* 1875, No. 47.
[10] Brattler, *Ein Beitrag zur Urologie*, München, 1858.
[11] Haxthausen, *Diss.* Halle, 1860.
[12] *Loc. cit.*

urinary phosphates, and not to actually increased excretion; a careful analysis of the twenty-four hours' urine should always be made. In true phosphaturia, of which the chief symptoms are nervous irritability and digestive troubles, the amount of P_2O_5 in the twenty-four hours may rise to 7 to 9 grammes.[1]

Calculi consisting wholly of calcium phosphate are exceedingly rare; uric acid calculi, however, are often covered with a coating of phosphates; the presence of the stone in the bladder sets up inflammation, the urine is thus rendered alkaline and calcium phosphate is precipitated.

OTHER INORGANIC SUBSTANCES

Iron occurs in small quantities; the compound in which it is present is unknown (Hamburger[2]).

Traces of silicic and nitric acids,[3] derived from drinking water, have been found.

Traces of fluorine are sometimes present.

Free ammonia occurs in mere traces also, but is increased when putrefaction sets in.

Hydrogen peroxide was found in traces in fresh urine by Schönbein.[4]

Sulphuretted hydrogen develops in putrid urine, probably not from the sulphates, but from other combinations of sulphur, such as sulpho- (thio-) cyanic acid,[5] and cystin (*see* Cystinuria, Chap. XLIV); hyposulphites may occur in typhoid fever urine.[6]

***The gases.*—The following table represents the chief analyses that have been made. The numbers are volumes per cent.:—**

Gases	Planer[7]	Pflüger[8]	Ewald[9]	Strassburger[10]
	Human urine			
Carbonic acid free .	4 to 9	13 to 14	Higher in fever than in health	The pressure of $CO_2 = 9{\cdot}15$ of an atmosphere, which is higher than in the blood
" " combined . . .	2 to 5	0·1 to 0·7		
Oxygen . . .	0·2 to 0·6	0·07 to 0·08	0·04	
Nitrogen . . .	0·7 to 0·8	0·8 to 0·9	0·9	

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[1] Tessier, *Du diabète phosphatique*, Lyons, 1877.

[2] *Zeit. physiol. Chem.* ii. 191; iv. 248.

[3] Röhmann, *Zeit. physiol. Chem.* v. 94.

[4] *Sitzungsber. d. Bayer. Akad. d. Wiss.* vol. i. (2), 1864, p. 115.

[5] Munk, *Arch. f. path. Anat.* lxix. 354; Gschleidlen, *Pflüger's Archiv*, xiv. 401; xv. 350.

[6] Müller, *Chem. Centralbl.* 1887, p. 807; *Berlin. klin. Woch.* xxiv. 405.

[7] *Zeit. d. Gesellsch. d. Aerzte in Wien*, 1859, p. 465.

[8] *Pflüger's Archiv*, ii. 165.

[9] *Arch. f. Anat. u. Physiol.* 1873, p. 1.

[10] *Pflüger's Archiv*, vi. 93.

CHAPTER XLIV

ABNORMAL AND PATHOLOGICAL URINE

Morbid conditions of the urine are exceedingly numerous. The urine may contain excess or diminution of one or other of its normal constituents. These conditions have been already described in the preceding chapters ; we may thus have urea, urates, phosphates and other salts in greater or less abundance than usual. The alterations in the pigments of the urine have already been described.

We have, however, now to consider alterations in the urine, in which substances normally absent from, occur to a greater or less extent in, that secretion. We shall also have to take up in a rather more connected way than we have done hitherto the deposits that occur in the urine.

The substances which occur in the urine under abnormal conditions are those introduced into the body with food or in the form of drugs ; and those which are due to the presence of disease of the urinary tract or other parts ; among these blood, pus, bile, albumin, and sugar are the most important.

It will be convenient to describe the heterogeneous group of cases we have to consider in the following order :—

1. Substances that appear in the urine as the result of the administration of drugs.
2. Deposits of various kinds that may occur in the urine.
3. Urinary stones, or calculi.
4. Blood and blood-pigment in the urine.
5. Bile in the urine.
6. Proteids in the urine.
7. The urine in diabetes.
8. Glycuronic acid in the urine.
9. Fats in the urine (chyluria)
10. Alkaptonuria.
11. Alkaloids in the urine.

DRUGS IN THE URINE

Inorganic salts.—Iodide, bromide and chloride of potassium or sodium, appear in great measure unchanged in the urine. Salts of cæsium, rubidium, lithium, and thallium behave similarly, as also do nitric, boric, and chloric acids.

Compounds of arsenic and antimony and lead pass only in slight amount into the urine. The excretion of lead is increased by the use of potassium iodide.

Mercury and silver, and other heavy metals, pass into the urine in mere traces, or after prolonged administration.

The alkalis and their carbonates pass into the urine, diminishing its acidity or making it alkaline. Acids combine in the body with bases, and pass into the urine as salts. Iodine appears as an iodate, sulphur as a sulphate.

Organic substances.—*Alcohol* when taken in excess appears in the urine only in traces. *Chloral* appears as urochloralic acid (Jaffe[1]); *chloroform*, partly as urochloralic acid, and partly is decomposed increasing the amount of chlorides. *Vegetable acids* are, as a rule, changed into carbonates. *Gallic* and *pyrogallic acids* are partly excreted as such, partly as pyrogallol, pyrocatechin, and other substances, which turn brown on exposure to the atmospheric oxygen in alkaline urine. *Tannin* appears chiefly as gallic acid; *benzoic acid* and allied benzoyl compounds combine with glycocine to form hippuric acid. The fate of *other aromatic substances* has been already described (Chapter XL.). *Quinine*, *strychnine*, and *morphine* are excreted for the most part unchanged, though sometimes morphine may be totally destroyed in the organism.

URINARY DEPOSITS

The different deposits that may occur in urine are chemical substances and formed elements.

The chemical substances are uric acid, urates, calcium oxalate, cystin, leucine, tyrosine, xanthine, phosphates, and indigo crystals.

The formed or anatomical elements consist of blood-corpuscles, pus, mucus, epithelium cells, spermatozoa, casts, fungi, and entozoa.

The methods of examining urinary deposits are partly chemical, partly microscopical. We can recognise the chemical substances by their characteristic reactions; the microscope even here comes to our aid, for it enables us to see whether the deposit is crystalline or amorphous, and, if the former, the shape of the crystals is often diagnostic. In the recognition of the anatomical elements, the microscope is the principal method of examination, chemical tests being of secondary importance.

In the examination of urinary deposits, it is important to note whether the urine contains the deposit immediately after being passed, or whether the sediment forms subsequently ; for instance, in the case of concentrated urine, the cooling that occurs after it leaves the bladder is often sufficient to cause a deposit of urates.

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[1] *See* Glycuronic Acid in Urine (p. 793).

When urine is allowed to stand for any length of time after being passed, one of two species of fermentation may occur. (1) *The alkaline fermentation.* Urea is changed into ammonium carbonate by the action of the *micrococcus ureæ* ; the ammonium carbonate is easily decomposed into ammonia and carbonic acid : this causes the urine to become alkaline, by which means the earthy phosphates are precipitated, and triple phosphate (coffin-lid crystals, fig. 99) is formed. Acid ammonium urates (fig. 95) may also be precipitated in alkaline urine. This fermentation may occur within the bladder in cases of catarrh of that organ, but under these circumstances the deposit of phosphates is mixed with excess of mucus, epithelium, or, in extreme cases, pus. (2) *The acid fermentation.* The deposition of urates is often accelerated by what is termed the acid fermentation, in which the acidity of the urine increases : this seems to be brought about by another fungus. The deposit consists chiefly of amorphous acid sodium urate ; crystals of uric acid and of calcium oxalate may also occur. A crystalline calcium phosphate ($CaHPO_4 + 2H_2O$) (Stein) may sometimes occur in acid urine (*see* p. 762). This fact is of some importance, as it accounts for the presence of calcium phosphate in or around uric acid calculi, even though the urine may have been acid throughout all the time that the stone was forming. The increase of fatty acids in certain cases of disease has been already alluded to, and is called lipaciduria (*see* p. 756). Hippuric acid is in decomposing alkaline urine often split into benzoic acid and glycocine ; perhaps this is also brought about by a bacterial growth.

Chemical Deposits in Urine

The following paragraphs give the principal facts in relation to the chemical substances that may occur in urinary sediments.

Uric acid.—A sandy, reddish deposit resembling cayenne pepper. It may be recognised by its crystalline form (fig. 93, p. 728), and the murexide reaction. The presence of these crystals generally indicates an increased formation of uric acid (*see* p. 733).[1] Voit and

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[1] Pfeiffer (*Seventh Congress of German Physicians*, Wiesbaden, 1888) brought forward a test for discovering excess of uric acid in the urine even when no deposit occurs. The twenty-four hours' urine is divided into two parts ; one of these is filtered through a filter on which pure uric acid is placed ; the other is not so treated. Equal volumes of each portion, say 100 c.c., are then acidulated with hydrochloric acid, and set aside. The precipitates of uric acid which form in both are collected on weighed filters, washed, and weighed. If the urine is normal, the yield in the two cases is about equal. On the other

Hofmann[1] consider that it arises from the decomposition of acid sodium urate.

Urates.—A deposition of urates may be due to their increased formation, to great concentration of the urine (as in fever), or to occurrence of the acid fermentation. They are tinted a brick-red colour by uroerythrin. They are generally amorphous; the acid urates of sodium and ammonium may be crystalline (figs. 94 and 95). They dissolve up on warming the urine to the temperature of the body. They may be collected by filtering them off from the urine; they will be found soluble in soda, from which solution crystals of uric acid form some hours after acidification with hydrochloric acid. Like uric acid, they give the murexide reaction. For Sir W. Roberts' views regarding the precipitation of uric acid and urates, *see* p. 731.

Calcium oxalate occurs in envelope crystals or dumb-bells; it is insoluble in ammonia and in acetic acid, soluble with difficulty in hydrochloric acid.

Cystin ($C_3H_6NSO_2$) is recognised by its colourless six-sided crystals (fig. 37, p. 86); these occur only in acid urine. They are easily soluble in ammonia, the caustic alkalis, and in mineral acids; insoluble in water, alcohol, ether, and dilute acetic acid; cystin is almost insoluble, though not absolutely so, in normal acid urine[2]; it dissolves when the urine is made alkaline.

A trace of sodium nitro-prusside, added to an alkaline solution of cystin, gives a violet colour.

A drop of lead acetate, added to a solution of cystin in caustic alkali, gives, when the mixture is boiled, a black precipitate of lead sulphide;[3] or if the solution be boiled in a silver dish, a black spot of silver sulphide is formed.

The origin of cystin in the body is unknown; Stadthagen[4] states that cystin is absent from normal urine. Goldmann and Baumann[5] succeeded in separating minute quantities of it from normal urine as a benzoyl compound. It may, however, occur in the urine without any other evidence of disease, and curiously enough cystinuria (i.e. cystin in the urine) runs in families. The chief danger arising from this condition is the formation of calculi, either in the bladder or kidney.

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hand, in gouty persons or those subject to uric acid gravel, the portion passed through the uric acid filter deposits its uric acid on the filter, so that on subsequent treatment with hydrochloric acid, little or no uric acid is obtained from it. Sir W. Roberts (*Lancet*, vol. i. 1890, p. 9), who has carefully examined the test, finds the results varying and inconstant, and therefore of little value.

[1] *Zeit. Anal. Chem.* vii. 397.

[2] Mester, *Zeit. physiol. Chem.* xiv. 109.

[3] There appear to be different isomerides of cystin which differ in the readiness with which they give up their sulphur (Goldmann and Baumann, *Zeit. physiol. Chem.* xii. 254).

[4] *Abst. Chem. Soc. Journal*, 1885, p. 830.

[5] *Loc. cit.*

On comparing the formula given above with that given for cystin on p. 86, it will be seen that the two are different. The more recent researches of Baumann have shown, however, that the formula $C_3H_6NSO_2$ is the correct one, or probably this empirical formula must be doubled to give the true molecular weight ($C_6H_{12}N_2S_2O_4$). Cystin is lactic acid in which H is replaced by NH_2 and OH by SH. In normal metabolism in the course of the formation of sulphuric acid products, a substance akin to cystin, and called cystein ($C_3H_7NSO_2$), is formed; the formation of cystin from cystein is an abnormal step in metabolism; and this Delépine considers is brought about by the action of a torula-like organism.[1] For Diamines in Cystinuria *see* Chap. XIII.

Leucine and tyrosine generally occur together.[2] The only conditions in which they are found in any appreciable quantity in the urine are acute yellow atrophy of the liver and phosphorus-poisoning. They have been also described in the urine of small-pox and typhus patients. They may be recognised by their crystalline form,[3] and by the tests which have already been described (p. 83), after their separation from the urine, which may be performed as follows: Precipitate the urine with lead acetate and filter. Pass sulphuretted hydrogen through the filtrate, to remove the lead; filter, and evaporate the filtrate to a syrup. Impure leucine and tyrosine crystallise out, and may be separated by hot alcohol, which dissolves the leucine, leaving the tyrosine in the residue.

Xanthine under rare circumstances occurs as a urinary deposit, or even forms stones. It may be recognised by its lemon-shaped crystals, insoluble on heating, insoluble in acetic acid, soluble in caustic potash. When evaporated with nitric acid, and the residue touched with caustic potash solution, it turns red, and on being heated reddish violet.

Phosphates.—The chief forms of phosphates that occur in urinary sediments are:—

(1) Calcium phosphate, $Ca_3(PO_4)$; amorphous.

(2) Triple phosphate, ($MgNH_4PO_4$); coffin lids (fig. 99, p. 762) and feathery stars.

(3) Crystalline phosphate of calcium, $CaHPO_4$, in rosettes of prisms, in spherules, or dumb-bells.

(4) Magnesium phosphate, ($Mg_3(PO_4)_2 + 22\ H_2O$), occurs occasionally and crystallises in long plates.

All these phosphates are dissolved by acids, such as acetic acid, without effervescence. A solution of ammonium carbonate (1 in 5) eats magnesium phosphate away at the edges; it has no effect on the triple phosphate. A phosphate of calcium, ($CaHPO_4 + 2H_2O$), may occur in acid urine.

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[1] *Proc. Roy. Soc.* xlvii. 198.

[2] *Oxymandel acid* ($C_8H_8O_4$) was observed to be present in acute yellow atrophy with leucine and tyrosine by Schultzen and Riess (*Chem. Centralbl.* 1869, p. 680). It was found in the ether extract of the urine after acidulation with sulphuric acid. It forms colourless, glistening crystals, with melting-point 162° C., sparingly soluble in cold water, easily in hot water, alcohol, and ether. It yields phenol on distillation with lime.

[3] The impure crystals as seen in the urine are generally yellowish spherical masses.

Calcium carbonate, $CaCO_3$, appears but rarely in deposits as whitish balls or biscuit-shaped bodies. It dissolves in acetic or hydrochloric acid with effervescence.

Indigo crystals are occasionally found in stale urine, especially in cases of cholera and typhus.

The following is a summary of the sediments of a chemical nature that may occur in urine :—

UNORGANISED SEDIMENTS IN URINE.—

IN ACID URINE

Uric acid.—Whetstone, dumb-bell, or sheaf-like aggregations of crystals, deeply tinged by pigment (fig. 93).

Urates of sodium, potassium, and ammonium.—Generally amorphous. The acid urate of sodium (fig. 94) and of ammonium (fig. 95) may sometimes occur in star-shaped clusters of needles, or spheroidal clumps with projecting spines. Tinged brick-red. Soluble on warming.

Calcium oxalate.—Octahedra, so-called envelope crystals (fig. 98). Insoluble in acetic acid.

Cystin.—Hexagonal plates (fig. 37). Rare.

Leucine. — Yellowish spheroidal clumps of crystals (fig. 32). Rare.

Tyrosine.—Bundles of silky needles (fig. 33). Rare.

Calcium phosphate, ($CaHPO_4 + 2H_2O$); *see* p. 762.

IN ALKALINE URINE

Phosphates.—

Calcium phosphate, $Ca_3(PO_4)_2$. Amorphous.

Triple phosphate, $MgNH_4PO_4 + 6H_2O$. Coffin lids (fig. 99) or feathery stars.

Calcium hydrogen phosphate. $CaHPO_4$. Rosettes, spherules, or dumb-bells.

Magnesium phosphate, $Mg_3(PO_4)_2 + 22H_2O$. Long plates.

All soluble in acetic acid without effervescence.

Calcium carbonate, $CaCO_3$.—Biscuit-shaped crystals. Soluble in acetic acid with effervescence. Rare.

Acid ammonium urate, $C_5H_2(NH_4)_2N_4O_3$. Thorn apple spherules (fig. 95).

Leucine and tyrosine.—Very rare.

Anatomical Elements in Urinary Sediments

These are as follows : –

(1) *Blood-corpuscles*, red and white.—Much of the hæmoglobin is dissolved by the urine, especially if that secretion is alkaline. Blood is recognised by the microscope and spectroscope. The red bi-concave discs generally become spherical, except when the urine is concentrated, in which case they become crenated. (*See* further Blood in Urine.)

(2) *Pus.*—This forms a white sediment resembling that of phosphates, and is frequently mixed with phosphates. The pus-corpuscles are indistinguishable from white blood-corpuscles. The addition of 1 per cent. acetic acid is generally necessary to render the nuclei

visible. Pus occurs in the urine as a result of suppuration in any part of the urinary tract (urethritis, gonorrhœa, cystitis, pyelitis, abscess in the kidney, tubercular kidney). Occasionally, especially after gonorrhœal inflammation of the prostate, white strings, made up of united pus-corpuscles, may appear in the urine. Some of the proteid constituents of the pus-cells, and the same is true for blood, pass into solution in the supernatant urine, and when the urine is boiled after faintly acidulating with acetic acid, it becomes cloudy from the formation of coagulated proteid.

On the addition of liquor potassæ to the deposit of pus-corpuscles, a ropy, gelatinous mass is obtained (Donné's test).

(3) *Mucus.*—This occurs to a small extent in normal urine, in excessive amount in catarrh of the urinary passages, and is generally mixed with pus when that is present in the urine. To the naked eye it appears as loose, flocculent masses; under the microscope it is mostly amorphous, together with a greater or less amount of epithelial cells, according as the catarrh is severe or mild.

It disappears, to a great extent, on the addition of caustic potash, because mucin, its chief chemical constituent, is soluble in alkalis. By this reaction it may be readily distinguished from pus.

(4) *Epithelial cells.*—These are of various kinds, according to the situation of the disease; cells from the renal tubules, from the pelves and ureters, from the bladder and urethra. They are larger than pus-cells, their nucleus is apparent without treatment with acetic acid, and their form is characteristic of the situation from which they come (*see* Chapter XXI, Epithelium). Cells from cancerous and other growths of the urinary tract may occasionally be found in the urine.

(5) *Spermatozoa* may be found if semen has passed into the urethra, or bladder, or into the vagina.

(6) *Casts.*—These moulds of the renal tubules only occur in urine in kidney disease, and the urine containing them is albuminous. They may be classed as follows :—

a. Hyaline casts, produced by the escape of blood-plasma into the tubules; this coagulates, and is carried into the main urinary stream by the pressure of fluid behind. If the epithelium of the tubule has been previously removed, larger hyaline casts are obtained.

b. Epithelial casts.—These are hyaline casts to which the detached epithelium of the tubule adheres.

c. Blood-casts.—These are casts formed in tubes into which hæmorrhage has occurred. They contain entangled blood-corpuscles, or may sometimes appear to consist wholly of blood-corpuscles.

d. Granular casts.—These are casts containing the *débris* of degenerated epithelium cells, or of blood-corpuscles; in the latter case they are yellowish.

e. Fatty casts.—These are casts dotted with fat-granules or globules, either within epithelial cells or free, i.e. after the disintegration of epithelial cells.

f. Albuminoid casts.—These are casts formed of waxy or albuminoid material which form in waxy, lardaceous, or albuminoid degeneration of the kidney. They are stained brown by iodine.

g. Mucus casts.—Casts of mucus, often of great length, sometimes occur, and do not necessarily indicate disease.

h. Seminal casts.—Casts of the seminal tubes of the testes. These contain spermatozoa.

i. Leucocyte casts.—These are formed of adherent leucocytes, and occur in suppuration, which involves the renal tubules.

j. Urate casts.—Acid sodium urate occasionally takes the form of a cast of the tubule in which it was excreted. This does not necessarily indicate disease. It dissolves on the application of heat.

(7) *Parasites.*—Healthy urine is free from microbes. They may be introduced from without, as by the use of dirty catheters ; they may arise from disease, such as tuberculosis of the urinary passages ; or they may even come from more distant parts, passing into the urine from the blood, as in anthrax, or carried by the blood to the kidneys in diphtheria, typhoid fever, ulcerative endocarditis, and other diseases. Sarcinæ and saccharomyces have been found in diabetic urine. Hooklets of *Tænia echinococcus*, ova of the fluke *Bilharzia hæmatobia*, embryos of *filaria sanguinis hominis*, and thread-worms from the vagina in women are instances of entozoa occurring in the urine.

Many of the preceding deposits occur but rarely in the urine. The following scheme for the preliminary examination of a urinary deposit takes into account only the commoner forms of sediment. It is also necessary to recognise in such a scheme that proteids, especially serum-albumin in solution in the urine, may occur in conjunction with a deposit.

I. Ascertain the reaction of the urine by means of litmus paper.

II. Note the naked eye appearance of the deposit, and perform the following tests :—

Remove some of the deposit from the bottom of the urine glass with a pipette, and shake it up with some of the supernatant urine	Naked eye appearance of deposit	Confirmatory tests to be applied to the sediment removed from the urine by filtration	Reaction of urine
a. Fill a test-tube half full of the turbid urine and heat			
i. The turbidity disappears URATES	Brick-red deposit	Murexide test	As a rule acid
ii. The deposit remains almost insoluble - URIC ACID	Cayenne pepper	Murexide test	Acid
iii. The turbidity increases - CALCIUM CARBONATE	White deposit	Dissolves in acetic acid with effervescence	Alkaline
EARTHY PHOSPHATES	White deposit	Dissolves in acetic acid without effervescence	Neutral or alkaline as a rule
ALBUMIN		Does not dissolve in acetic acid	Acid or alkaline

Remove some of the deposit from the bottom of the urine glass with a pipette, and shake it up with some of the supernatant urine	Naked eye appearance of deposit	Confirmatory tests to be applied to the sediment removed from the urine by filtration	Reaction of urine
β. Half fill another test-tube as before, and add a drop of acetic acid			
i. The turbidity disappears — EARTHY PHOSPHATES			
ii. The turbidity increases — MUCUS (sometimes) iii. It remains unaltered — MUCUS (sometimes)	Loose, flocculent deposit	Add caustic potash; the sediment partly or wholly dissolves	usually alkaline
If these are present the urine will be albuminous: PUS	White deposit	Add caustic potash; a stringy gelatinous mass forms	
BLOOD	Red deposit	Examine spectroscopically (*see* Blood in Urine)	Acid or alkaline
CALCIUM OXALATE	White deposit	Dissolves in hydrochloric acid	Acid

III. Remove a small amount of the deposit by a pipette, mount it on a glass slide, cover with a cover-slip, and examine for crystals, blood-corpuscles, pus-corpuscles, urinary casts, epithelium-cells, bacteria, &c.

URINARY CALCULI

Concretions, called sand, gravel, and stones, according to their size, may form in the pelvis of the ureter, the ureter, sinus of the prostate, but most frequently in the bladder. They may result from the conglomeration of urinary deposits, when they are said to be *primarily* formed ; or the deposition may occur around some foreign body, such as small blood-clots, or pieces of pencil, broken ends of catheters, or other material introduced into the bladder from without ; these are said to be *secondarily* formed. The deposition in all cases occurs in concentric layers around a central nucleus ; in primary stones this is most frequently uric acid, or calcium oxalate, or both ; in secondary stones, the foreign body forms the nucleus.

After what has been already said concerning urinary sediments, it is only necessary to give a brief notice of the different forms of stones. The substances deposited in alkaline urine will be phosphates ; in acid urine, uric acid, urates and calcium oxalate. If the reaction of the urine changes during the formation of the calculus, alternate layers of these two sets of materials will be found.

The following are the principal varieties of stones that occur :—

(1) Calculi, composed of uric acid or urates, with either little or no admixture with phosphates. They are generally reddish and smooth, sometimes tuberculated. They form about three-fifths of the total number of stones operated on.

(2) Mixed calculi : stones like the preceding, but containing a

larger quantity of phosphates than of uric acid, usually in concentric layers, alternating with uric acid, or forming a coating on the surface of a uric acid nucleus. This variety comprises nearly two-fifths of the remaining cases.

(3) Calcium oxalate calculi : these are dark brown or grey, very hard, generally tuberculated, when they are called 'mulberry calculi'; if smooth, they are termed 'hemp-seed calculi.' They comprise about 3 per cent. of the total number of stones that occur.

(4) Phosphatic calculi : a calculus composed of pure calcium phosphate is exceedingly rare.[1] A nucleus of uric acid is generally present. The phosphates deposited are principally calcium phosphate, triple phosphate, or a mixture of calcium and magnesium phosphates. This last named is the commonest; it is often soft, like chalk, and melts under the blowpipe flame, being therefore called the fusible calculus.

(5) *Calcium carbonate calculi* are those generally found in the prostate.

(6) *Cystin calculi* are mostly small, smooth, and have a yellow tinge, changing to green when kept in the air. These are rare.

(7) *Xanthine calculi* : these are still rarer.

(8) *Fibrinous calculi* (composed of fibrin or inspissated albumin) have a glassy appearance on fracture. *Blood-calculi* have been described in cases of renal hæmaturia. *Uro-stealith calculi* are probably composed of fatty material.[2] *Indigo calculi* : only one has yet been recorded (Ord).

The following is a scheme for the analysis of calculi.[3] It is best to scrape off and powder a little from each layer and examine it separately.

SCHEME FOR ANALYSIS OF URINARY CALCULI

Burn a little of the powder on a piece of platinum foil over a spirit-lamp or Bunsen flame.

A. It burns away completely, or leaves a mere trace of residue.

(1) There is a smell of burning feathers during the process. **ALBUMIN, FIBRIN, BLOOD.** Confirm by proteid tests.

(2) It first melts on heating, giving off an aromatic smell. Fresh portions are found : (*a*) soluble in ether, (*b*) in caustic potash. **URO-STEALITH.**

(3) It gives off purple red vapour. A fresh portion dissolves in sulphuric acid with a blue colour, showing spectroscopically a band before D. **INDIGO.**

The above are all rare. Generally there is no odour, and no coloured fumes. The stone then consists of uric acid, ammonium urate, cystin, or xanthine. The two last named are also rare. Take a fresh portion of the powder, and digest with warm dilute hydrochloric acid.

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[1] Sir Henry Thompson has only operated on one case (*Clinical Lectures*, 5th edit. p. 67).

[2] *Heller's Archiv*, 1844–5; Moore, *Dublin Quart. Journ.* 1854.

[3] Adapted from Salkowski and Leube.

a. *It dissolves completely.*

CYSTIN or XANTHINE

Dissolve a fresh portion in ammonia and filter; evaporate off the ammonia from the filtrate; hexagonal crystals of cystin form. The crystals may be also obtained by adding acetic acid to the ammoniacal solution.

Dissolve a fresh portion in nitric acid; evaporate to dryness on a porcelain dish; add caustic potash when cool; a red colour is produced, which becomes reddish violet on heating.

b. *It dissolves incompletely.*

Filter; wash the residue with water. Try the murexide reaction. Both uric acid and ammonium urate stones give this reaction. To test for ammonia proceed as follows: Warm a fresh portion of the powder with sodium carbonate solution. Ammonia is given off, and may be recognised by its smell, blueing of red litmus paper, and fumes with a glass rod wetted with hydrochloric acid. If ammonia is present, the stone consisted of AMMONIUM URATE; if absent, of URIC ACID.

B. The powder becomes black, owing to the organic substances present, but leaves a considerable residue. The stone may then consist of urates of sodium and potassium, or earthy phosphates, or calcium oxalate, or carbonate.

(1) Take a fresh portion and place on platinum foil. It melts under the blowpipe flame. FUSIBLE CALCULUS. Confirm by dissolving in dilute hydrochloric acid and testing for phosphates.

(2) Take a fresh portion of the powder; add dilute hydrochloric acid.

a. *It dissolves completely.*

Uric acid is absent.

If it dissolves with effervescence, CALCIUM CARBONATE is present. If there is no effervescence, it is absent.

If it dissolves without effervescence, and a fresh portion of the powder be incinerated, and then effervesces with hydrochloric acid, the stone contains or consists of CALCIUM OXALATE, which, by the process of incineration, had been converted into the carbonate.

If it dissolves without effervescence either before or after incineration then the portion of stone taken consists wholly of PHOSPHATES. If, however, there is effervescence, phosphates are not necessarily absent; they may be mixed with the carbonate, or more rarely with the oxalate of calcium, which gave the effervescence before and after incineration respectively. Phosphates may be tested for by the nitro-molybdate and other well-known tests. The metals calcium and magnesium may be tested for in the usual way (p. 717).

b. *It dissolves incompletely.*

Uric acid is present, and may be tested for in the residue in the manner described above (A b). The stone then consisted of URATE OF SODA. Traces of that of potash may be also present.

Phosphates, calcium carbonate, or calcium oxalate may have been mixed with the urate, but these pass into solution in the dilute hydrochloric acid used; these may be tested for as described in the accompanying column (a). Sodium and potassium, calcium and magnesium also pass into solution if present as chlorides. These metals may be detected as already described (p. 717).

Stones often contain a small percentage of *iron*. This also passes into the hydrochloric-acid solution; render it feebly alkaline with ammonia; cool; acidulate with acetic acid; yellowish white flocculi of ferric phosphate separate out. Collect these on a filter, dissolve them in hydrochloric acid, add ferrocyanide of potassium and Prussian blue is developed.

BLOOD AND BLOOD-PIGMENT IN URINE

When hæmorrhage occurs in any part of the urinary tract, blood appears in the urine. Blood which comes from the kidneys, as in acute Bright's disease, cancer of the kidney, scurvy, purpura hæmorrhagica, endemic hæmaturia (due to the parasite *Bilharzia*), and after the use of turpentine, cantharides, and other drugs, is generally mixed uniformly with the urine. Blood which comes from the bladder is often clotted, and usually comes in greatest abundance towards the termination of the act of micturition, while that from the urethra stains most deeply the urine that is passed at the commencement of micturition. Blood from the prostate is generally uniformly distributed in the urine, so resembling blood from the kidneys.

If a large quantity of blood is present, the urine is deep red, especially if it is alkaline ; the microscopic examination of the sediment reveals the presence of blood-corpuscles ; on spectroscopic examination. the bands of oxyhæmoglobin are well seen.

If only a small quantity of blood is present, and is uniformly mixed with the urine, that secretion has a characteristic reddish-brown colour, which clinical observers have termed 'smoky.' This is especially seen when the urine is acid. The precise cause of the smoky tint has received but scant attention ; it may be in some cases merely due to admixture of the oxyhæmoglobin and urinary pigments ; more often it is due to the formation of methæmoglobin. Methæmoglobin is generally formed in small quantities in acid urine which contains blood after its removal from the body. In other cases still, the pigment occurs in a condition more akin to hæmatin[1] than to hæmoglobin. In some cases very little of the blood passed, dissolves in the urine ; the most characteristic spectrum which is then obtainable from the deposit is that of hæmochromogen.[2] The deposit collected on a filter is dissolved in rectified spirit containing ammonia, and a drop of ammonium sulphide added. The two characteristic bands of hæmochromogen then appear (*see* fig. 59, spectrum 9, p. 277).

Another test for blood that may be applied is the formation of hæmin crystals (p. 291). The guaiacum test is wholly untrustworthy, and should be altogether discarded.

Hæmoglobinuria and methæmoglobinuria.—The blood-pigment may under certain circumstances appear in the urine without the presence of any blood-corpuscles whatever. That this was the case was first shown by Pavy. Sometimes brown masses, composed of granular pigment, looking like casts as they were moulded in the urinary tubules, are seen in these cases. This condition is produced by a destruction of blood-corpuscles in the circulation[1]; the hæmoglobin so liberated behaves as hæmoglobin solution does when injected into the blood-stream, passing into the urine probably *via* the Malpighian glomeruli. The injection of substances into the blood-stream, which have a solvent effect on the red corpuscles, acts in the same way; such substances are glycerin, solutions of bile-salts, distilled water, or the injection of the blood of one animal into that of another. The same result follows poisoning with arseniuretted hydrogen, hydrochloric, sulphuric, carbolic, and pyrogallic acids, phosphorus, potassium chlorate, &c.; and in the diseases pyæmia, typhus, scurvy, fat embolism, after severe burns, and in some cases of jaundice there is also occasionally hæmoglobinuria.

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[1] Lewin and Posner, *Centralbl. med. Wiss.* 1887, No. 20; MacMunn and Armitage. *Brit. Med. Journ.* ii. 1888, p. 120.

[2] MacMunn, *Brit. Med. Journ.* July 19, 1879.

One of the most interesting, however, of all these conditions is a disease known as '*paroxysmal hæmoglobinuria*'; a disease which resembles ague in the occurrence of periodical febrile attacks, but is also accompanied by the presence of blood-pigment in the urine.

The next question before us is, what is the condition of the blood-pigment in the urine in these cases? It is never free from admixture with methæmoglobin; but if the amount of pigment is large, oxyhæmoglobin is present as well. The urine in these cases is generally acid, but the change into methæmoglobin is apparently not the result of the action of the urine on the pigment, but of the kidney-cells during the act of excretion.

Hoppe-Seyler[2] and MacMunn[3] state that in every specimen from the paroxysmal form of the disease, methæmoglobin is present, and Hoppe-Seyler has gone so far as to say that oxyhæmoglobin is always absent, and to suggest that the name 'paroxysmal methæmoglobinuria' would be more correct. I have myself had opportunities of examining several specimens of the urine in different fits from seven cases of the disease. In three of these, methæmoglobin was the only pigment present, and the urine was brownish red; in all these cases the total amount of pigment was small. In the other four cases, methæmoglobin alone was present at the commencement of the attack; in a few hours, as the attack progressed, the quantity of pigment increased, and oxyhæmoglobin appeared as well. The spectroscopic appearances of such a specimen, when examined in a shallow layer, are a faint band between C and D, but nearer C, due to methæmoglobin (fig. 59, spectrum 6), and the two typical bands of oxyhæmoglobin, in addition, between D and E. On adding a drop of potassium ferricyanide to this, the methæmoglobin band becomes darker, the oxyhæmoglobin bands disappear, and are replaced by the two fainter bands, due to methæmoglobin, which have approximately the same position as the oxyhæmoglobin bands. The urine, during this change, turns browner. If one adds a drop of ammonium sulphide solution[1] instead of potassium ferricyanide, the methæmoglobin band fades, the oxyhæmoglobin bands get a little darker, and then are rapidly replaced by the single band of reduced hæmoglobin (fig. 59, spectrum 3).

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[1] When the destruction occurs in the portal circulation, however, hæmoglobinuria does not occur (Hunter, *Lancet*, vol. ii. 1888, pp. 515, 618, 654).

[2] *Physiol. Chem.* p. 862.

[3] *Clin. Chemistry of Urine*, p. 164.

In one case, Neale[2] found oxyhæmoglobin crystals in the urine. In some cases bile-pigments have been found, in addition to the blood-pigment[3]; in other cases they are absent.[4]

On boiling the urine of paroxysmal hæmoglobinuria, a brown coagulum is formed. This is due to the coagulation of globin, the proteid constituent of hæmoglobin. In three of the cases I have examined, serum-albumin was present in addition, and in one of these serum-albumin occurred in the urine at the commencement of the attack, previous to the appearance of blood-pigment in that secretion.[5]

BILE IN URINE

Bile appears in the urine in the condition known as icterus, or jaundice. The varieties of jaundice and their causes have been already described in connection with the blood (p. 311) and the bile (p. 688).

Bile-pigments in urine give it a greenish-yellow, or greenish-brown, colour, which in severe cases may approach black. In two cases bilirubin crystals have been found in the urine.[6] Urine containing excess of urobilin may simulate icteric urine, and so the following tests should always be performed :- -

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[1] The urine must always be examined immediately after ammonium sulphide has been added, for in a few minutes it becomes cloudy and opaque.

[2] *Lancet*, vol. ii. 1879, p. 725.

[3] Hoppe-Seyler, *Loc. cit.*; Kühne, *Virchow's Archiv*, xiv. 319; Herrmann, *Diss.* Berlin, 1859; Tarchanoff, *Pflüger's Archiv*, ix. 53.

[4] Naunyn, *Arch. f. Anat. und Physiol.* 1868, p. 402; Steiner, *Ibid.* 1873, p. 160.

[5] This case was under Dr. Ringer's care in Univ. Coll. Hosp. and is recorded by Barton, *Clin. Soc. Trans.* 1890, p. 30. Foulerton (*Lancet*, ii. 1890, p. 709) is inclined to consider that the not infrequent association of hæmoglobinuria with oxaluria is not merely accidental.

[6] Hoppe-Seyler and v. Recklinghausen noted the appearance of bilirubin crystals in the urine of a boy into whose vessels lamb's blood had been transfused; and Ebstein (*Deutsch. Arch. klin. Med.* 1878, p. 115) found similar crystals in urine which contained blood in a case of pyonephrosis.

(1) *Gmelin's reaction.*—This test consists in pouring the urine on to the surface of some nitric acid in a test-tube; the play of colours, green, blue, red, yellow, is produced at the junction of the two liquids. The test may be also performed on a white plate; a drop of nitric acid placed in the centre of a film of the urine on the plate becomes surrounded with coloured rings if bile-pigments are present. The spectroscopic appearances of these colour-changes are characteristic (p. 683), and may thus serve to distinguish bile in doubtful cases from other pigments which give certain colours with nitric acid. Ordinary urine always becomes rather darker on the addition of nitric acid from oxidation of its chromogens; urine containing excess of skatoxyl pigment (p. 745) becomes red; urine containing excess of indoxyl sulphate of potassium becomes blue or violet from the formation of indigo.

Gmelin's test is so delicate and so perfectly characteristic that it is seldom necessary to separate out the pigments. This, however, may be done by Hoppe-Seyler's method as follows[1]: Add milk of lime to the urine, and pass a stream of carbonic acid through the mixture till no further precipitation takes place. The precipitate carries down with it the bile-pigment, if present, while urobilin and indican are left in solution. The precipitate is collected; a little water and a little chloroform acidulated with acetic acid are added, and the mixture shaken; biliverdin colours the water green, and bilirubin colours the chloroform yellow. Both solutions give Gmelin's reaction.

(2) *Maréchal's test.*[2]—A few drops of tincture of iodine (B.P.) are carefully poured on to the surface of the urine in a test-tube. If bile-pigments are present a green colour appears below the red layer of iodine tincture. If the urine is very dark it should be diluted with water before applying the test.

The Bile-salts.—Vogel and Dragendorff[3] found 0·8 gramme of these salts in 100 litres of normal urine. In other words, they are for practical purposes absent from normal urine. It is also very difficult to detect them in icteric urine, even if the jaundice be due to obstruction of the bile-duct[4]. This is not so difficult to understand if it be remembered that Frerichs, after injection of the bile-salts into the circulation, found only small quantities in the urine.

It is never possible, speaking from my own experience, to detect bile-salts in the urine direct by means of Pettenkofer's test. They must always be separated out: 100 to 200 c.c. of the jaundiced urine are evaporated to dryness on the water-bath; the residue is extracted with absolute alcohol; the extract is filtered, and twelve or twenty times its bulk of ether added. This precipitates the bile-salts; the precipitate is collected, dissolved in water, decolourised by animal charcoal, and then Pettenkofer's reaction tried with this solution; a drop of strong solution of cane sugar is added to the solution of bile-salts in a capsule; a drop of sulphuric acid is added to this, and the capsule gently warmed; a rich purple colour is developed (*see* more fully p. 681). Excess of sugar or excess of sulphuric acid must be avoided, or else a yellow brown or black colour, due to charring, is produced.

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[1] *Physiol. Chem.* p. 864.

[2] *Journ. de Pharm. et de Chim.* March 1869; *see* also W. G. Smith, *Dublin Journ. Med. Science*, 1876, p. 452.

[3] *Zeit. anal. Chem.* ii. 467.

[4] In a recent paper on this subject Baelde and Lavrand (*Compt. rend. soc. biol.* v. 629) state they found bile-acids in the urine in all cases of jaundice, but give no details as to method.

Two additional tests may be tried with urine suspected of containing bile-salts: (1) a solution of acid-albumin is prepared; a few drops of the icteric urine added to this; the albumin is precipitated. True peptone is not precipitated by bile-salts, as is sometimes erroneously stated. (2) Sublimed sulphur sinks in urine containing bile-salts, as the latter lower the surface tension of fluids (Hay).

PROTEIDS IN THE URINE

We have already seen that in health, although urine is formed partly by filtration processes, it is free from proteids. The condition sometimes called 'physiological albuminuria' is really pathological or abnormal; the abnormal condition is, however, slight and temporary. If it becomes exaggerated and permanent, as in diseases of the kidney, it is a most serious condition, for, besides the interference with kidney function, there is a constant drain of nutrient matter from the body.

The proteids of the blood are four in number; one of these, hæmoglobin, and its appearance in the urine have been already described; another, fibrinogen, passes into the urine in only one disease, namely, chyluria (which *see*). If fibrinogen passes through the renal cells in Bright's disease, it seems to be immediately converted into fibrin, forming the renal casts already mentioned. The remaining two, serum-albumin and serum-globulin, are those most frequently found in cases of albuminuria; and, as a general rule, serum-albumin is present in greater abundance than the globulin.

Proteids are present in solution in cases of hæmaturia (blood in the urine) and pyuria (pus in the urine), and also when semen is mixed with the urine. In these cases very often there is albuminuria in addition; that is, the proteid coagulable by heat is more abundant than is accountable for by a small admixture with blood, pus, or semen.

Other proteids are sometimes found in the urine in addition to those which exist normally in blood; thus egg-albumin may be present after a too liberal diet of eggs. It may be distinguished from serum-albumin by the fact that it is precipitated by ether, while serum-albumin is not. Proteoses are present in some diseased conditions; peptone or mixtures of peptone and proteoses in others.

So-called 'physiological albuminuria.'—The most marked condition in which this occurs is after prolonged muscular exercise. Leube[1] found albumin in the urine in 16 per cent. of soldiers after a prolonged march ; Chateaubourg[1] gives the percentage as even higher ; Oertels,[2] on the other hand, places it at 3 per cent. The tests applied in these cases (especially in those of Chateaubourg) were not wholly satisfactory, and the subject demands reinvestigation. It is, however, probable that vigorous muscular action, especially in those at all prone to kidney disease, may produce a temporary congestion of that organ and lead to temporary albuminuria. A somewhat similar condition sometimes occurs after the application of cold to the body,[3] as after a cold bath. The blood is driven into the interior of the body from the skin, and the renal vessels are thus over-filled. In some cases, again derangements of the nervous system (which interfere with the vaso-motor-nerve regulation of the kidney vessels), and derangements of digestion, and anæmia (which alter temporarily the composition of the blood), may lead to a similar temporary or functional albuminuria.

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[1] *Virchow's Archiv*, lxxii. 145; lxxix.; *see* also Marcazzi, *Gaz. hebdom.* 1879, No. 16.

Experimental albuminuria.—The following experimental interferences with the kidney circulation are followed by the appearance of the serum-proteids in the urine :—

(1) Pressure upon (not closure of) the renal veins[4] ; the pressure in the glomeruli is thus increased.

(2) Closure of the renal artery, and subsequent re-establishment of the circulation : this is due to interference with the nutrition of the renal cells.

(3) Ligature of the aorta below the one kidney, and extirpation of the other. This raises the pressure as in (1).

(4) Ligature of the aorta above the giving off of the renal arteries.

(5) Compression of the trachea : this leads to asphyxia and the consequent rise in blood-pressure.

We thus see that conditions of increased blood-pressure in the kidneys lead to albuminuria. Runeberg,[5] from a curious misunderstanding of some of his experiments, stated that lessened blood-pressure produced this result. His mistake was demonstrated by Gottwalt.[6]

Under the heading of experimental albuminuria must also be included the appearance of egg-albumin in the urine after its injection into the vessels of animals, or after too great ingestion of eggs both in animals and men.

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[1] *Recherches sur l'albuminurie physiologique*, 1883 ; *see* also Senator, *Albuminuria in Health and Disease*, New Syd. Soc. translation, 1884 ; Saundby, *Glasgow Med. Journ.* June 1884 ; Ralfe, *Diseases of the Kidney*, p. 588.

[2] *Ziemssen's Handbuch der allgemein. Therapie*, iv.

[3] Lassar, *Arch. path. Anat.* vol. lxxix. 1880.

[4] Perls, *Arch. f. exper. Pathol.* vi. 118.

[5] *Arch. d. Heilk.* xviii. 1 ; *Deutsch. Arch. f. klin. Med.* xxiii. 41, 225.

[6] *Zeit. physiol. Chem.* iv. 428.

Albuminuria in disease.—The pressure of tumours or of the pregnant uterus on the renal veins will cause albuminuria, as in the experiments just mentioned. Venous congestion in heart disease will act in the same way. In certain conditions of the blood, albumin appears to pass more readily than normally through the renal cells, and appears in the urine ; e.g. in anæmia (this is perhaps partially explicable by the lessened nutrition of the renal cells) ; in the first stage of convalescence from cholera, just when the blood after being viscous and almost at a standstill in the kidneys, is becoming more fluid again. Phosphorus-poisoning, and the excessive use of morphia, and the poisons of certain diseases cause albuminuria; this is especially seen in scarlet fever, which may be followed in bad cases by Bright's disease: to a less extent in typhoid, diphtheria, and pneumonia. It also may occur in diabetes.[1]

The most frequent cause of albuminuria is, however, Bright's disease, in which the kidneys are actually diseased, and may even cease work altogether, producing uræmia (p. 315). The forms of Bright's disease are—

(*a*) Acute Bright's disease. Much albumin, and often blood.

(*b*) Large white kidney ; a fatty degeneration of the kidney-cells. Much albumin as a rule.

(*c*) Granular contracted kidney; an overgrowth of connective tissue pressing on the renal tubules. Much urine secreted ; quantity of albumin smaller than in (*a*) and (*b*).

(*d*) Albuminoid kidney. Albuminoid or waxy degeneration of the kidney substance. Quantity of urine increased; quantity of albumin comparatively small.

In Bright's disease renal casts can generally be discovered in the urine. The amount of albumin in urine rarely exceeds 1 per cent.: it may, however, rise as high as 4 per cent. (Hoppe-Seyler).[2] The word albumin has been used, as is usual with clinical observers, as the name of the proteid in urine. It should, however, be remembered that the proteid serum-globulin is nearly always present as well. The relation of albumin to globulin in the blood and lymph is termed the proteid-quotient (p. 341). If this is high or low, the proteid-quotient in the urine is high or low also respectively.[3] The relation of albumin to globulin in urine has not been much investigated, for, so far as we know at present, the matter is one of mere theoretical interest. The practical point a physician wishes to ascertain is whether or not there is in the urine a proteid which is coagulated by heat. Both albumin and globulin are precipitated on heating the urine, the size of the coagulum giving a rough indication of the amount of proteid present.

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[1] Maguire, *Brit. Med. Journ.* vol. ii. 1886, p. 543.

[2] *Physiol. Chem.* p. 858.

[3] Pigeaud, Hoffmann, *Maly's Jahresb.* xvi. 474; Noel Paton, *Brit. Med. Journ.* i. 1890, p. 196.

In order to ascertain whether or not *serum-globulin* is present, the urine must be neutralised, and then saturated with magnesium sulphate. A precipitate indicates the presence of serum-globulin.[1] This precipitate may be collected on a filter, dissolved by the addition of water. The solution so formed coagulates at 75° C. The albumin coagulates about the same temperature, but is found, not in the precipitate produced by magnesium sulphate, but in the filtrate from which that precipitate has been removed.[2]

When a large quantity of serum-globulin is present, the following test of Sir W. Roberts may be applied: A glass vessel is filled with water, and a few drops of the urine allowed to fall into it. Each drop leaves a milky trace behind it, and when a number of drops have been added, the water becomes opalescent. On adding acetic acid it again becomes clear.

Occasionally serum-globulin is found without albumin in the urine; sometimes it exceeds the serum-albumin in quantity. Usually the serum-albumin is in excess. Senator finds that the quantity of globulin is greater in waxy degeneration of the kidneys than in other forms of Bright's disease.[3] In severe organic disease of the kidney, and in the albuminuria that occurs in diabetes, Maguire[4] finds the proportion albumin : globulin = 2·5 : 1 a common one.

In testing for these substances, the account already given of the proteids in Chapters X and XV should be consulted. The following list of tests may, however, be found useful, as it embodies all the chief reactions by which albuminuria is discovered. Towards these tests, albumin and globulin behave alike. If the urine is cloudy, it should be filtered first. The best and most trustworthy test is undoubtedly the first in the following list; it is, moreover, the simplest.

Proteoses in urine.—An albumose was first described in urine in a case of osteomalacia by Bence Jones.[5] It has been since found in the urine in this disease by Kühne[6] and others. Hoppe-Seyler[7]

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[1] Some albumoses are also precipitated by this salt, but give certain ether special tests to be mentioned later.

[2] Mr. F. Smith (Army Veterinary Department) recently pointed out to me that normal urine gives a precipitate on saturation with magnesium sulphate. What this precipitate consists of is at present unknown; it is not proteid in nature.

[3] Noel Paton (*loc. cit.*) was not able to confirm this observation.

[4] *Brit. Med. Journ.* vol. ii. 1886, p. 543.

[5] *Phil. Trans.* vol. i. 1848, p. 55.

[6] *Zeit. Biol.* xix. 209.

[7] *Physiol. Chem.* p. 858.

Test	Reaction	Remarks
(1) Heat to 73° to 75° C., or boil; the top of a long column of urine in a test-tube should be heated; the difference between this and the clear urine below is then well seen	The proteid is coagulated	If the urine is alkaline it must be acidulated with a few drops of weak acetic acid, either before or after boiling. The precipitate which is formed is insoluble in acetic acid, and can thus be distinguished from phosphates. Nitric acid should not be used in this test, as coagulated albumin is slightly soluble in nitric acid
(2) Add nitric acid	The proteid is precipitated. (Occasionally in very concentrated urine a crystalline precipitate of urea nitrate is produced, but this is most exceptional)	On subsequently boiling, this precipitate either does not dissolve at all or only slightly
(3) Heller's nitric acid test. The urine is poured gently on to the surface of some nitric acid in a test-tube	A ring of white precipitate at the junction of the two liquids is seen	This test is especially applicable to urines containing only a small quantity of albumin
(4) Johnson's picric acid test. A concentrated solution of picric acid is poured on to the surface of the urine in a test-tube	A ring of white precipitate occurs at the junction of the two liquids; this increases on heating	This method of applying the test is applicable to urines containing a small amount of albumin. The test may be also performed by mixing the two reagents together, or by adding a little solid picric acid to the urine. A precipitate forms in both cases. Peptones and albumoses are precipitated by this reagent, but the precipitate redissolves on heating
(5) Potassio-mercuric iodide (Tauret's reagent [1]) added to the urine	Causes a white precipitate	This reaction may also be performed in Heller's method. This reaction is the most delicate of the various reagents investigated by a committee of the Clinical Society.[2] It is, however, inferior to Heller's nitric-acid test, as it precipitates peptone and albu-

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[1] Mercuric chloride, 1·35 gramme; potassium iodide, 3·32 grammes; acetic acid 20 c.c., water 64 c.c.

[2] *Clin. Soc. Trans.* xix. 339.

Test	Reaction	Remarks
		mose (this precipitate, however, redissolves on heating), alkaloids, and also bile-salts (these may be extracted from the precipitate by ether)[1]
W. Roberts' dulated brine ce of hydro- id to a pint of sodium chlo- ion) added to	Causes a white precipitate of the proteid	
ier precipitants ls, e.g. sodium with or with- : acid, ferro- of potassium, phoric acid, ɔeen from time commended		For convenient bed-side testing these reagents are sometimes used in the solid form. Pavy's pellets consist of citric acid and potassium ferrocyanide. Papers previously soaked in potassio-mercuric iodide, potassium ferrocyanide, sodium tungstate, and, lastly, picric acid have been prepared by Dr. Oliver. The Clinical Society's committee report that of these, potassio-mercuric iodide papers are the best

in several cases of atrophy of the kidneys, Lassar[2] in the people rubbed with petroleum, Oertel[3] in a few cases after ertion. Both it and peptone are found in the urine of animals se circulation they have been introduced.[4]

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ier proteose sometimes found in the urine is deutero-pro- ich closely resembles peptone in its reactions, and is often for or mixed with peptone. It will be more appropriately d in connection with peptonuria.

:, *Compt. rend. soc. biol.* (8), iv. 869.

sen's *Handbuch d. Therapie*, 1884.

path. Anat. iv. 309.

[2] *Arch. path. Anat.* lxxvii. 104.

[4] Neumeister, *Zeit. Biol.* xxiv. 272.

[6] *Zeit. Biol.* xxii. 409.

Test	Reaction	Remarks
(1) Heat to 73° to 75° C., or boil; the top of a long column of urine in a test-tube should be heated; the difference between this and the clear urine below is then well seen	The proteid is coagulated	If the urine is alkaline be acidulated with a fe of weak acetic acid, eithe or after boiling. The tate which is formed is in in acetic acid, and can t distinguished from phos Nitric acid should not b in this test, as coagulate min is slightly soluble in acid
(2) Add nitric acid	The proteid is precipitated. (Occasionally in very concentrated urine a crystalline precipitate of urea nitrate is produced, but this is most exceptional)	On subsequently boili precipitate either does n solve at all or only slight
(3) Heller's nitric acid test. The urine is poured gently on to the surface of some nitric acid in a test-tube	A ring of white precipitate at the junction of the two liquids is seen	This test is especially cable to urines containi a small quantity of albu
(4) Johnson's picric acid test. A concentrated solution of picric acid is poured on to the surface of the urine in a test-tube	A ring of white precipitate occurs at the junction of the two liquids; this increases on heating	This method of appl test is applicable to ur taining a small amount min. The test may be formed by mixing th agents together, or by little solid picric ac urine. A precipitate both cases. Peptone moses are precipita reagent, but the pre dissolves on heating
(5) Potassio-mercuric iodide (Tauret's reagent[1]) added to the urine	Causes a white precipitate	This reaction ma formed in Heller's r reaction is the mo the various reagent by a committee o Society.[2] It is, ho to Heller's nitric· precipitates pept

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[1] Mercuric chloride, 1·35 gramme; potassium iodide, 3·32 gramm water 64 c.c.

[2] *Clin. Soc. Trans.* xix. 339.

	Reaction	Remarks
		mose (this precipitate, however, redissolves on heating), alkaloids, and also bile-salts (these may be extracted from the precipitate by ether)[1]
N. Roberts' lated brine of hydro- to a pint of sodium chlo- de) added to	Causes a white precipitate of the proteid	
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Hetero-proteose (whether it be hetero-albumose or hetero-globulose), if it occurs alone in the urine, may be detected in the following way:—

1. Heat the urine to 65°. A precipitate forms in neutral or faintly alkaline urine, which, unlike coagulated albumin or globulin, dissolves in a few drops of dilute hydrochloric acid. This precipitation does not occur in acid urine.

2. Add nitric acid to the urine; a precipitate forms which dissolves on heating and reappears on cooling.

3. Add a drop of dilute copper-sulphate solution and excess of potash; a rose-red colour forms (biuret reaction). Albumin and globulin give a violet colour.

4. Saturation with magnesium sulphate causes precipitation.

5. Picric acid, potassio-mercuric iodide, mercuric chloride (in acid solution), sodium tungstate, all give white precipitates.

If a proteose is present mixed with peptone, saturation with ammonium sulphate precipitates the former and leaves the latter in solution.

If proteoses or peptones, or both, are present mixed with albumin and globulin, heating the urine (after acidulation if the urine is alkaline) precipitates the latter and leaves the two former in solution. As, however, there is risk of the formation of a small amount of primary proteoses (proto- and hetero-) by the hydrating action of the acidified hot liquid, a better method of separation consists in adding to the urine ten or twelve times its volume of absolute alcohol. A precipitate of all the proteids is produced; by the end of two or three weeks the alcohol renders albumin and globulin insoluble; the proteoses and peptones are soluble, and may be dissolved out with distilled water.

The properties of proteoses, and tables for their separation from one another, and from other proteids, will be found fully given in Chapter X. A table for the separation of the various proteids that occur in the urine will be found at the end of this section.

Peptonuria.—There appear to be many conditions in which peptone has been described in the urine.[1] In healthy urine no peptone is present. In decomposing albuminous urines it may be formed from the albumin after the urine is passed. Many of the observations that have been made on this subject were published previous to the appearance of Kühne and Chittenden's recent work on proteoses and peptones, and many of the older experiments therefore now require careful revision. It is only within the last few years that we have been furnished with accurate data for the identification and separation of these substances. The proteid most liable to be mistaken for peptone is deutero-proteose. They may be readily distinguished and separated from one another by saturating the urine (slightly acidified with acetic acid) with ammonium sulphate; this salt precipitates the deutero-proteose, and leaves peptone in solution. Any proteid that remains in solution after filtering off the precipitate produced by thorough saturation with ammonium sulphate must be peptone. This is, in fact, the only method by which peptone can be identified[2] with certainty. A solution of peptone so obtained gives no precipitate with nitric acid, nor with copper sulphate; it is not precipitated by heating; it is precipitated by alcohol, but not coagulated by that reagent. It is also precipitated by tannin, potassio-mercuric iodide, phospho-molybdic acid, phospho-tungstic acid, and picric acid. It gives a well-marked xantho-proteic reaction (yellow colour on boiling with nitric acid, turned orange or brown by ammonia), and biuret reaction (rose-red colour with copper sulphate and caustic potash).

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[1] It was first described in urine by Gerhardt, *Deutsch. klin. Arch. Med.* v. 215.

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Martin has found that most of the so-called cases of peptonuria (especially in purulent diseases) are really cases of deutero-proteose in the urine. The following table contrasts the behaviour of these two substances in various reactions :—

Deutero-albumose	*Peptone*
(1) Gives no precipitate with nitric acid unless a considerable amount of salt be also added. This precipitate disappears on heating, and reappears on cooling.	(1) Gives no precipitate with nitric acid under any circumstances.
(2) Is precipitated by saturation with ammonium sulphate.	(2) Is not precipitated by saturation with ammonium sulphate.

In all other respects these two substances behave similarly.

The following are the conditions in which peptonuria has been described: In phosphorus-poisoning,[1] in suppurative diseases, and croupous pneumonia[2] (probably derived from the peptone that forms in decomposing pus and exudations, *see* p. 363); in acute rheumatism, typhoid fever, typhus fever, small-pox, scarlet fever, mumps, tuberculosis, erysipelas, empyema (another suppurative disease), cancer of the liver and intestines, catarrhal jaundice, parametritis, apoplexy, &c.

When injected into the blood, peptone rapidly appears in the urine. When deutero-albumose is injected into the blood, it appears in the urine as peptone; this is seen especially in carnivorous animals, whose urine is rich in pepsin. Urine rich in pepsin, however, produces no further digestive action when mixed with proteoses; first, because free acid is absent, and, secondly, because many of the salts of the urine exert an inhibiting influence on the ferment. The change probably occurs in the act of secretion, where there is a momentary occurrence of free acid (Neumeister[3]).

The following table gives the method for separating serum-albumin, serum-globulin, hetero-proteose, deutero-proteose, and peptone should they happen to be all present in the urine together. Such an occurrence is very rare; but in doubtful cases it is best to test for every one in the list.

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1. If the urine gives no precipitate on boiling after acidulation, albumin and globulin are absent. If a precipitate occurs, albumin or globulin or both are present.

2. If the urine after neutralisation gives no precipitate on saturation with magnesium sulphate, globulin and hetero-proteose are absent. If such a precipitate occurs, one or other is present.

3. If the urine be saturated with ammonium sulphate and filtered, and the filtrate gives no xanthoproteic or biuret reaction (a large excess of potash must always be added), peptone is absent.

4. If the urine gives no precipitate on boiling after acidulation, no precipitate with nitric acid, and no precipitate on adding ammonium sulphate to saturation peptone can be the only proteid present. Confirm this by the biuret reaction.

5. If all proteids are present they may be separated as follows: –

Saturate the urine (faintly acidified with acetic acid) with ammonium sulphate. A precipitate is produced. Filter.

a. *Precipitate*	b. *Filtrate*
Contains albumin, globulin, hetero- and deutero-proteose.	Contains peptone.

Collect the precipitate on a filter, wash it with saturated solution of ammonium sulphate, and redissolve it by adding a small quantity of water. To this solution add ten times its volume of alcohol; a precipitate is formed; collect this, and let it stand in absolute alcohol for from seven to fourteen days. Then filter off the alcohol, dry the precipitate at 40° C., extract it with water, and filter. An insoluble residue is left.

a. *Residue*	b. *Extract*
This consists of albumin and globulin coagulated by the alcohol.	This contains the proteoses in solution.

Hetero-caseose is precipitated by heating the solution to 65° C., or by saturating a portion of the extract with magnesium sulphate. Deutero-proteose remains in solution.

Take another portion of urine, neutralise it, and saturate with magnesium sulphate. A precipitate is produced. Filter.

a. *Precipitate*	b. *Filtrate*
This consists of globulin and hetero-proteose, which may be separated by the prolonged use of alcohol, as above.	This contains albumin, deutero-proteose, and peptone. Add alcohol as above; albumin is rendered in seven to ten days insoluble in water. The deutero-proteose and peptone are soluble, and may then be separated by ammonium sulphate.

THE URINE IN DIABETES

Diabetes insipidus is a disease in which there is a very abundant secretion of very watery urine, and is probably dependent on a derangement of the vaso-motor nerves of the kidney or their centre. The disease which, however, we have now to deal with is called, in contradistinction to the above, *diabetes mellitus*, and is characterised by a very abundant secretion of urine[1] with a high specific gravity

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[1] The quantity in the twenty-four hours rarely falls below two litres; it may rise to eight and even ten litres.

(1030 to 1050), and containing dextrose. Sugar is present in both blood and urine normally in traces. The excess that occurs in this disease in both fluids, passing from the blood into the urine probably at the glomeruli, is due to a disordered state of the metabolic functions of the liver or of the muscles.[1] Injury to the floor of the fourth ventricle produced artificially in animals (Bernard [2]), or by disease in man, produces glycosuria, probably by interference with the centre of the vaso-motor nerves of the liver. Complete extirpation of the pancreas also produces glycosuria (*see* p. 663).

Transitory glycosuria (sugar in the urine) may occur in cholera, ague, cerebro-spinal meningitis, liver cirrhosis, and gout. Certain poisons (morphia, curare, chloroform, &c.) were said formerly to produce glycosuria; we now know that the substance in the urine in these cases which reduces Fehling's solution is not sugar, but glycuronic acid. The presence of other substances in the urine besides sugar that reduce Fehling's solution is a great source of error when only a small amount of reduction occurs: these substances are uric acid, hippuric acid, creatinine, pyrocatechin, and glycuronic acid. The fermentation test is the best means of distinguishing sugar from these other bodies. Sugar alone is transformed into alcohol and carbonic acid under the influence of yeast. In addition to dextrose, urine may in this disease contain small quantities of inosite (p. 100), of levulose,[3] and in a few cases glycogen itself has been found (Leube [4]).

Milk sugar, which occurs often in the urine of nursing mothers, is also apt to be mistaken for grape sugar. It undergoes the alcoholic fermentation slowly, and can only be distinguished with certainty from grape sugar by separating it out from the urine and examining its properties (*see* p. 757). In these cases, however, the symptoms of diabetes are absent.

The quantity of grape sugar in diabetic urine is, as a rule, over 4 per cent., rising to 7, 9, or even 12 per cent. The quantity varies considerably with the diet; carbohydrate diet increases it; the withdrawal of carbohydrate food, especially if combined with the administration of morphia or codria, diminishes it, or may even cause the entire disappearance of sugar from the urine. In the most severe form of diabetes, however, both drugs and dieting are useless.

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[1] The reader will find an account of recent researches on this question in Bunge's *Physiol. Chem.* translated by Wooldridge. It, however, appears to me that Bunge is inclined to lay too much stress on the muscular glycogen as a source of diabetic sugar. *See* also pp. 814 and 540 *et seq.* of this book. Phloridzin diabetes referred to on p. 544 has been the subject of several researches since that page was written. The chief memoirs on the subject are: v. Mering, *Verhandl. V. and VI., Cong. inn. Med. Zeit. klin. Med.* xiv. 415, xvi. 431. Moritz and Prausnitz, *Zeit. Biol.* xxvii. 81; Külz and Wright, *Ibid.* p. 181.

[2] *Leçons*, Paris, 1855, pp. 288, 355.

[3] For references *see* p. 120; to these may be added, Külz, 'On lævorotatory sugar in urine,' *Zeit. Biol.* xxvii. 228.

[4] *Virchow's Archiv*, cxiii. 391.

The properties and reactions of dextrose have been already fully dealt with in Chapter IX; it is, therefore, only necessary here to briefly recapitulate the tests which are most suitable for its detection in urine.

1. The copper test.—Boiling with Fehling's solution (which should itself be previously boiled to ensure that no reduction occurs in it without admixture with urine) produces a yellow or red precipitate of the cuprous hydrate or oxide. Trommer's test (the addition of copper sulphate to the urine followed by caustic potash) does not in my experience answer so well.[1]

Fehling's solution in solid form may be applied to urine by means of Pavy's test-pellets, and Dr. Oliver has introduced cupric test-paper.

2. Bismuth test.—This consists in the black precipitate of metallic bismuth that occurs on boiling an alkaline solution of bismuth nitrate with the urine. For this purpose Bottger's solution (bismuth subnitrate 5 grammes, tartaric acid 5 grammes, 30 c.c. distilled water, and strong caustic soda added carefully till a clear solution is obtained), or Nylander's reagent (bismuth subnitrate 2 grammes, sodio-potassium tartrate 4 grammes, liquor sodæ 55 c.c., distilled water 47 c.c.) may be employed.

As albuminous materials in the urine may also produce a black precipitate from the formation of bismuth sulphide, Brücke[2] recommends the following method: Fröhn's reagent is made as follows: freshly precipitated bismuth subnitrate, 1·5 gramme, and 20 c.c. water are heated to boiling, and 7 grammes potassium iodide and 20 drops of hydrochloric acid are then added. Equal quantities of urine and water are put into two test-tubes; hydrochloric acid is added to the water till Fröhn's reagent no longer produces cloudiness. In this way the necessary quantity of hydrochloric acid is ascertained, and this quantity is added to the urine; the reagent is then added and the mixture filtered. The filtrate should not now become cloudy on adding either hydrochloric acid or the reagent. It is boiled with excess of caustic soda or potash; if a grey or black colour results sugar is present.

3. Moore's test.—The urine containing sugar is heated with caustic potash solution, and the mixture becomes yellow, then brown.

4. Picric acid test.[3]—Heat the urine with a few drops of concentrated solution of picric acid, or a little solid picric acid may be used. Add caustic potash, and a brown red colour, due to puramic acid, is obtained.

5. Fermentation test.—A test-tube is half filled with the urine, and a little German yeast added; the tube is filled up with, and inverted over mercury, and left in a warm place for twenty-four hours. Carbonic acid collects in the tube, and may be tested for in two ways: (1) it is wholly absorbed by strong caustic potash solution; (2) it gives a white cloudy precipitate with lime or baryta-water. The liquid gives the tests for alcohol.

A control experiment should be made in another test-tube with yeast and water, as a small yield of carbonic acid is often obtained from impurities in the yeast.

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[1] *See* also Lauder Brunton, 'Non-precipitation of Cuprous Oxide in certain cases of Diabetic Urine' (*Bartholomew's Hosp. Reports*, xvi. 235); a yellow solution instead of a yellow precipitate occasionally forms, the cuprous oxide being apparently held in solution by certain urinary constituents.

[2] *Wien. Akad. Sitzungsber.* vol. lxii. 1875, 2. Abth.

[3] G. Johnson, *Lancet*, November 18, 1882.

Another method of performing the test is to connect a flask containing the urine and yeast by a bent glass tube with another vessel containing lime-water; the gas passes into the second flask as it comes off, and gives a precipitate of calcium carbonate with the lime-water.

In order to separate grape sugar from the urine the latter is evaporated to a syrup on the water-bath, and the residue extracted with absolute alcohol; evaporate the extract to dryness, and once more extract with absolute alcohol; add to this extract a solution of potash in 80 per cent. alcohol; a precipitate forms which after pouring off the alcohol is dissolved in water, neutralised with acetic acid, and precipitated with lead acetate, filtered, and the filtrate treated with sulphuretted hydrogen; the lead sulphide is removed by filtration, and the filtrate contains the grape sugar, which crystallises out on evaporation, and may be purified by recrystallisation (Salkowski and Leube).

Another method consists in precipitating the urine with normal lead acetate, filtering, and treating the filtrate with basic lead acetate and ammonia. This precipitates the sugar; the precipitate suspended in water is decomposed with sulphuretted hydrogen and filtered. The filtrate is evaporated, and grape sugar crystallises out (Brücke).

Hydroxybutyric acid in the urine.—In addition to grape sugar, diabetic urine may contain other substances. One of these is an acid first discovered in the urine by Minkowski,[1] which he found to be identical with the *β-hydroxybutyric acid* of Wislicenus, but differing from it in being optically active; $(\alpha)_D = -23{\cdot}4$. Such acids are poisonous when introduced into the circulation. Its quantity in the urine is variable; it often occurs in the urine when acetone also is present, and diacetic acid, a substance allied to acetone, can be formed from it. Diacetic acid may also be found in the urine of diabetics; its quantity and that of the hydroxybutyric acid vary in a parallel degree (Wolpe[2]). The presence of β-hydroxybutyric acid in diabetic urine has been also observed by Külz[3] and by Stadelmann,[4] who give methods for its separation from urine, but we are not at present acquainted with the clinical significance of its appearance there.

Acetone and ethyl-diacetic acid in the urine.[5] The appearance of these substances in the urine is full of clinical importance.

Acetone, or dimethyl ketone (C_3H_6O), has been prepared artificially, and its chemical relationship to the alcohols has already been described (p. 66). It may be detected by the following tests:—

(1) In the pure state it forms, with excess of concentrated aqueous solution of sodium bisulphite, a crystalline compound (acetone + sodium bisulphite) which separates out in shining scales.

(2) Lieben's iodoform test, as modified by Ralfe, may be used to detect acetone in urine: 20 grains of potassium iodide are dissolved in a drachm of liquor potassæ and boiled: the urine is then carefully floated on to its surface in a test tube. At the point of contact a precipitation of phosphates occurs, which, if acetone is present, becomes yellow and studded with yellow points of iodoform. This test is much better obtained by distilling a small quantity of urine and applying it to

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[1] *Arch. exp. Path. u. Pharmak.* [illegible]

[2] *Zeit. Biol.* xx. [illegible]

[3] *Chem. Centralbl.* 1887, p. 277

[4] *Ibid.* p. [illegible]

[5] I am indebted for many of my references on this subject to a chapter with the above heading in MacMunn's *Clinical Chemistry of Urine*

the distillate. This test has the disadvantage that lactic acid and ethyl alcohol behave similarly.

(3) Le Nobel's test.[1]—On adding an alkaline solution of sodium nitro-prusside, so dilute as to have only a slight red tint, to a fluid containing acetone, a ruby-red colour is produced, which in a few moments changes to yellow, and on boiling, after adding acid, to greenish blue or violet. A quarter of a milligramme of acetone can be thus detected.

(4) Chautard's test.[2]—A drop of aqueous solution of magenta decolourised by sulphurous acid gives with fluids containing over 0·01 per cent. of acetone a violet colour. This appears in dilute solutions after the lapse of four or five minutes.

(5) Baeyer and Drewsen's indigo test.—A few crystals of nitro-benzaldehyde are dissolved by heat in the fluid suspected to contain acetone; on cooling, the aldehyde separates as a white cloud. The mixture is then made alkaline with dilute soda, and if acetone be present, first yellow, then green, followed by an indigo blue colour, appear within ten minutes.

In cases where only traces of acetone are present, large amounts of urine (50 litres) are acidulated with sulphuric acid, and submitted to fractional distillation, the lighter volatile part being collected. Acetone in the distillate is recognised by its boiling point (56° to 58° C.), specific gravity (0·814 at 0° C.), odour, and the reactions just mentioned. Alcohol, which is sometimes present in the urine with acetone, may also distil over. To separate them the residue is treated with fused calcium chloride in excess, and distilled on the water-bath; the distillate treated with calcium chloride and again distilled; the process may be again repeated; acetone distils over. Alcohol remains with the calcium chloride residues, which give it off by distillation over the free flame. It may be detected by the iodoform reaction and the formation of aldehyde and acetic acid on oxidation (Salkowski and Leube).

Ethyldiacetic acid ($C_6H_{10}O_3$) strikes a Bordeaux red colour with a solution of ferric chloride; it has often been confused with and mistaken for acetone; but the reaction with ferric chloride distinguishes them, and from this test its presence in urine is generally inferred.[3] Under the influence of alkalis it takes up water and splits into acetone, alcohol, and carbonic acid ($C_6H_{10}O_3 + H_2O = C_3H_6O + C_2H_6O + CO_2$).[4] If this occurs in the blood (for Acetonæmia, *see* p. 314) or urine it is probably the sodium salt, which undergoes a similar decomposition.

$$\underset{\substack{\text{[sodium ethyl}\\ \text{diacetate]}}}{C_6H_9NaO_3} + 2H_2O = \underset{\text{[acetone]}}{C_3H_6O} + \underset{\text{[alcohol]}}{C_2H_6O} + \underset{\substack{\text{[sodium hydrogen}\\ \text{carbonate]}}}{NaHCO_3}$$

This view of the origin of acetone was supported by the fact that alcohol was found in the urine with it. Some observers, however, have noted certain facts which bear against this theory of the origin of acetone. There are other substances that may occur in the urine which give the ferric chloride reaction, namely, β-hydroxybutyric acid, sulpho- (thio-) cyanates, acetic acid, and formic acid; and, according to Legal,[5] the urine of patients who have taken thalline, antipyrine, salicylic, and carbolic acids may also give it. If, however, the urine is previously boiled, diacetic acid, if present, still gives the ferric-chloride reaction, but these other substances do not. Fleischer[1] found that the substance which gives the ferric-chloride reaction in diabetic urine is not taken up by ether after the urine has been acidulated with sulphuric acid, whereas ethyl-diacetic acid is soluble in ether. Salkowski[2] confirmed this observation, and moreover found the urine after boiling did not give the ferric-chloride reaction. These observers, therefore, conclude that in most cases it is not ethyl-diacetic acid from which acetone originates, but some other at present unknown, but probably related substance; it may chance to be the hydroxybutyric acid already mentioned. Whatever the substance may be that causes it, the appearance of this reaction in diabetic urine is of grave import, often foretelling the onset of diabetic coma and death. This substance is, in fact, probably the poison which produces these effects. It appears certain that the poison is not acetone.[3] The smell of acetone in the breath and urine should always cause one to give a careful prognosis; but acetone may be absent and diabetic coma ensue; and acetone may be present without any sign of coma, or even of diabetes. v. Jaksch[4] found acetone in the urine of healthy people in amount up to 1 centigramme in the twenty-four hours. It is probably a product of normal metabolism. It is increased in diabetes, but also in many febrile conditions, e.g. small-pox, typhus, pneumonia, scarlet fever, measles, cancer, Bright's disease, perityphlitis, strangulated hernia, &c. and in these conditions no diabetic coma ensues. West has confirmed v. Jaksch on most points.[5] Acetone given in large doses to animals and men, even when diabetic, produces no coma (Salomon and Brieger[6]).

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[1] *Chem. Centralbl.* 1884, p. 626. Legal's test, *Journ. Pharm.* (5), xviii. 206, is almost the same as Le Nobel's.

[2] *Bull. soc. chim.* xlv. 83.

[3] This was first noted in urine by Gerhardt, *Wien. med. Presse*, 1871, No. 1.

[4] Rupstein, *Centralbl. med. Wiss.* 1874, No. 55.

[5] *Loc. cit.*

GLYCURONIC ACID IN THE URINE

Glycuronic acid ($C_6H_{10}O_7$) is the substance which, above all others, is liable to be mistaken for sugar in the urine. Uric acid, creatinine, and hippuric acid, even when in excess in the urine, rarely produce more than a small amount of reduction of Fehling's solution. But glycuronic acid gives a heavy yellow or red precipitate of cuprous hydrate or oxide in the same way as dextrose does. It also reduces bismuth, silver and mercury oxides in an alkaline solution, and is dextrorotary.

It occurs in normal urine in such small quantities that it may be considered to be practically absent. It occurs in the urine in abundance after the administration of certain poisons and drugs, such as chloral and butyl chloral, nitrobenzol, orthonitrotoluol, camphor, curare, morphia, and after chloroform narcosis. These drugs were, by the older observers, said to produce glycosuria; but that sugar is absent can be conclusively shown by the non-occurrence of the alcoholic fermentation with yeast. The only absolute means, however, of identifying glycuronic acid in the urine is to separate it out and examine its properties.

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[1] *Deutsch. med. Woch.* 1879, No. 18.

[2] *Die Lehre vom Harn*, p. 397.

[3] As Kussmaul supposed (*Deutsch. Arch. klin. Med.* vol. xiv. 1874). Acetone was first found in diabetic urine by Petters, *Prager Vierteljahrssch.* vol. lv. 1857.

[4] *Acetonurie und Diacetonurie*, Berlin, 1885; *see* also Deichmüller and Tollen, *Ann. d. Chem.* ccix. 22; Windle, *Liverpool Med. Chir. Journal*, July 1884; Saundby, *Birmingham Med. Review*, February 1885.

[5] West, *Med. Clin. Trans.* lxxii. 91.

[6] *Zeit. klin. Med.* vi. Heft i.

The chief properties of this substance with references to literature will be found on p. 110. We have now to consider the method to be adopted in separating it from urine, and to discuss its clinical significance.

The best method to obtain glycuronic acid from urines that contain it is that recommended by Schmiedeberg and Meyer.[1] A large quantity of urine is decolourised by animal charcoal, evaporated to a syrup, and then digested with large quantities of damp barium hydrate in the presence of gentle heat over a water-bath. It is then extracted with absolute alcohol; glycuronic acid and other substances are left undissolved. The residue is mixed with water and filtered; more baryta is added to the filtrate; it is again filtered, and the filtrate evaporated down over a water-bath. An amorphous barium compound separates out; this is washed with water, decomposed by sulphuric acid; the barium sulphate is filtered off, the filtrate is evaporated down and dried *in vacuo*, when crystals of the anhydride will be obtained.

There is some doubt as to the precise compound it forms in the urine. The principal salt present appears to be potassium glycuronate. Compounds with urea are also present, and there is little doubt that the substances described by Jaffe[2] and others as urochloralic acid, uronitrotoluol, &c., as occurring in the urine after the use of certain drugs are aromatic compounds of glycuronic acid. Indoxyl glycuronate, skatoxyl glycuronate, and other aromatic compounds have also been described. Its formation, according to Ashdown,[3] probably occurs in the renal secreting cells.

The clinical significance of the appearance of this substance in the urine after the use of drugs is not great. The condition of the urine is, in these cases, a transitory one, and to the physician it is a matter of merely theoretical interest whether the reducing substance in the urine is sugar or glycuronic acid. In certain cases, however, it appears in the urine without any drug treatment; to the physician and for purposes of life assurance it is then most important to be able to say whether or not diabetes is present. A case recorded by Ashdown of a man who otherwise was perfectly healthy is an illustration of this; and it may in the future be found that other supposed cases of diabetes are really cases of glycuronic acid in the urine. The latter condition does not appear to be dangerous to life, while diabetes is a most serious disease. In Ashdown's case the urine was not increased either in volume or density.

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[1] *Zeit. physiol. Chem.* iii. 422.

[2] *Ibid.* ii. 47. *See* also Külz, *Zeit. Biol.* xxvii. 247.

[3] *Brit. Med. Journ.* vol. i. 1890, p. 169.

FATS IN THE URINE

Fats may be present in the urine under three conditions :—

(1) When no disease of the kidneys is present.

a. From excess of fat in the food (Bernard,[1] Wiener,[2] Seriba [3]).

b. After administration of cod-liver oil (Sir W. Roberts [4]).

c. In fat-embolism occurring after fractures.

d. In the fatty degeneration of the liver that occurs in phosphorus-poisoning.

e. In cases of long-standing suppuration, phthisis, and pyæmia ; here doubtless fatty degeneration of the pus-cells occurs.

f. In diabetes mellitus, when there is often a lipæmic condition [5] (*see* p. 315).

In all these cases the excess of fat passes into the blood, and thence to the kidneys.

(2) In disease of the urinary organs.

g. In Bright's disease ; fatty casts are often seen.

h. In pyonephrosis (Ebstein [6]). This comes under the same category as the cases included under *e*.

(3) In a peculiar disease, which occurs in the tropics, known as *chyluria*. This is sometimes accompanied with the formation of tumours containing lymph, in the regions of the scrotum and thighs (*see* p. 349). Its most marked symptom, however, is the passing of milky urine. It is produced by a parasitic worm called the *filaria sanguinis hominis* ; this inhabits the lymphatics, especially of the scrotum and lower limbs. It is also found in large numbers in the kidneys. Not only chyle, but also blood may be found in the urine. In chylous urine the following abnormal constituents are found : Fibrinogen, serum-globulin, serum-albumin, finely divided fats, traces of soaps, lecithin, and cholesterin [7] ; in fact, all the constituents of chyle are present.[8] Chylous urine usually coagulates when passed or deposits strands of fibrin. It is difficult to explain the occurrence of chyluria without supposing that a fistulous communication exists between the lacteals and the urinary passages ; and though sometimes

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[1] *Leçons*, Paris, vol. ii. 1859, p. 86.

[2] *Arch. path. Anat.* ii.

[3] *Deutsch. Zeit. f. Chir.* xii. 118.

[4] Quoted by MacMunn, *Clin. Chemistry of Urine*, p. 203.

[5] Kobert, *Diss.* Halle, 1880.

[6] *Deutsch. Arch. f. klin. Med.* xxiii. 113.

[7] Cholesterin has also been found in the urine in fatty degeneration of the kidneys (Beale, *Archives of Medicine*, 1857), in diabetes and jaundice (Salisbury, *Amer. Journ. Med. Sciences*, 1863), and in the urine of an epileptic treated with potassium bromide (Pohl, *Petersburger med. Woch.* 1877, p. 171).

[8] For analyses of chylous urine *see* Hoppe-Seyler, *Physiol. Chem.* p. 870; Eggel, *Deutsch. Arch. klin. Med.* vi. 421; Brieger, *Zeit. physiol. Chem.* iv. 407.

a post-mortem examination has failed to reveal the presence of such, in other cases it has been found (Odennis and Lang,[1] Hensen [2]).

For the recognition and estimation of the fat, lecithin, and cholesterin, the same methods may be employed as have already been described in connection with nervous tissue (p. 533). They are all soluble in ether; the ether is evaporated off, and they are found in the residue. The characteristic reactions of fats are described on p. 487, of lecithin p. 526, and of cholesterin p. 531.

ALCAPTONURIA

We have already seen that, after the administration of carbolic acid, gallic acid, and other aromatic compounds, the urine if alkaline becomes dark-brown on exposure to the air. This condition is produced by the oxidation of pyrocatechin, hydroquinon, pyrogallol, and similar substances. The same compounds probably cause a similar darkening that occurs in the urine of herbivora, the diet of these animals containing a large amount of aromatic substances.

In certain cases, the pathology of which is not well understood, an excess of these same aromatic compounds appears in human urine without the administration of drugs ; the urine consequently, as in so-called carboluria, darkens on exposure to the air. Cases of this kind were first described by Bödeker,[3] and he called the substance alcapton, and the condition alcaptonuria. Gorup-Besanez [4] was the first to suggest that alcapton and pyrocatechin are the same thing. Salkowski and Leube, Epstein and Müller,[5] take the same view. W. G. Smith [6] and Preusse [7] believe that, in addition to pyrocatechin, protocatechuic acid is largely present in these cases. Udranszky [8] believes that both of these are derived from an organic compound, which he calls a humous substance. In some cases examined by Kirk,[9] he separated an acid from the urine, which he calls uroleucic acid ($C_9H_{10}O_5$), which, according to Huppert, is probably pyrogallolpropionic acid.

It is possible that in different cases we may have different aromatic compounds present; they all darken on oxidation; and they all reduce Fehling's solution, and must not be confounded with dextrose.

ALKALOIDS IN THE URINE

The absorption of alkaloids from the alimentary canal sometimes occurs ; these are carried to the kidneys and excreted there unchanged;

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[1] Virchow-Hirsch, *Jahresb.* vol. ii. 1874, p. 674.
[2] *Pflüger's Archiv*, x. 94.
[3] *Zeit. rat. Med.* vii. 128.
[4] *Lehrbuch*, ii. 324.
[5] *Virchow's Archiv*, lxii. 554.
[6] *Dublin Journ. Med. Sci.* Jan. 1882.
[7] *Zeit. physiol. Chem.* ii. 324.
[8] *Ibid.* xi. 537; xii. 33.
[9] *Brit. Med. Journ.* vol. ii. 1888, p. 232; vol. ii. 1889. p. 1149.

atropine, quinine, and strychnine, and sometimes morphine, may thus, after their administration to a patient, be found in his urine.

Whether ptomaines and leucomaines, formed by putrefaction in the intestine, pass normally in the same way into the urine is very doubtful. If such alkaloids as neurine and choline are thus formed, they are probably wholly destroyed by further putrefaction before there is time for absorption to take place (*see* Chapter XXXV).

In cases of disease, symptoms may be sometimes explained by supposing that absorption of poisonous animal alkaloids is occurring. That such is the case remains to be proved in most cases.

Normal urine is free from all alkaloids, except creatinine, and the same is true for most cases of morbid urine. Diamines have, however, been found in cases of cystinuria, cholera, and pernicious anæmia.

The toxicity of normal urine has been explained by Ponchet and Bouchard as due to the presence of ptomaines. Stadthagen has, however, shown that it is more probably due to the potassium salts the urine contains.

A fuller discussion of the subject with reference to literature will be found in Chapter XIII.

DIAZO-REACTION IN URINE

The diazo-reaction, sometimes called Ehrlich's reaction, is as follows: Two solutions are necessary; (1) a concentrated solution of sulphanilic acid; (2) a solution of sodium nitrite (1 in 200). 200 c.c. of (1) are mixed with 10 c.c. of pure hydrochloric acid and 6 c.c. of (2). Equal quantities of this mixture and the urine are mixed and rendered strongly alkaline with ammonia. A bright carmine-red colouration constitutes the reaction. After standing twelve to thirty-six hours, a deposit occurs, the upper part of which is green or black. The reaction is never given by healthy urine. Rutimeyer (*see* 'Lancet,' ii. 1890, p. 413), who has examined 260 urines, states it is of special value in the diagnosis of typhoid fever, as it is given by typhoid urine, but not in the urine from cases of intestinal catarrh. It is also given by the urine from cases of acute tuberculosis (due to the absorption of caseous matter?) and in certain other diseases. The exact diagnostic value of the test is a matter for future clinical research, and a good deal of correspondence on the subject will be found in the medical journals.

CHAPTER XLV

QUANTITATIVE ANALYSIS OF URINE

ESTIMATION OF THE TOTAL SOLIDS

a. The amount of total solids may be obtained approximately by calculation from the specific gravity. The last two figures of the specific gravity are multiplied by 2·33 (for adults),[1] or by 1·66 (for children). Thus, if a man passes in the day 1500 c.c. of urine with a specific gravity of 1021, then 21 × 2·33 = 48·93 grammes in 1000 c.c., or 73·39 grammes of solids in the day's urine (1500 c.c.)

b. A more accurate process is the following: Take 5 c.c. of urine in a weighed capsule. Evaporate it in the receiver of an air-pump by placing the capsule over a dish containing concentrated sulphuric acid. After twenty-four hours place fresh sulphuric acid in the dish, exhaust again, and weigh after another twenty-four hours. Deduct the weight of the capsule, and the remainder gives the total solids in 5 c.c. of urine.

c. A quicker process is to evaporate the urine in a weighed capsule to dryness over the water-bath, and then dry the residue by placing the capsule in an air-bath at 110° C. for a few hours. Cool in an exsiccator, and weigh. This method is not, however, very accurate, as some of the compounds in the urine decompose at this temperature.

d. In the residue the proportion of total organic to total inorganic substances may be ascertained by the processes described on p. 18.

ESTIMATION OF ACIDITY

a. Acidity is usually expressed in terms of oxalic acid.

The following solution is necessary:—

Standard caustic soda solution.—This contains 40 grammes of pure caustic soda in a litre of distilled water. This would exactly neutralise 63 grammes of pure oxalic acid: 1 c.c. will therefore neutralise an amount of acidity corresponding to 0·063 gramme of oxalic acid. This solution is then diluted to ten times its volume with water; 1 c.c. of this decinormal solution is equivalent to 0·0063 gr. of oxalic acid.

Method.—Take 50 c.c. of urine in a flask, and allow the alkaline solution to drop into it from a burette. After every addition of alkali shake the mixture well, and test its reaction by taking out a drop on a clean glass rod, and putting it on litmus paper. When the reaction is such that red litmus paper is blued, and blue paper reddened, the amount used from the burette is read off.

This amount multiplied by 0·0063 gives the amount of acidity in terms of oxalic acid in 50 c.c. of urine. This multiplied by 2 gives the percentage. If the urine is alkaline the amount of alkalinity can be similarly determined by means of a decinormal solution of oxalic acid.

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[1] 2·33 is the number given by Haeser and Christison, 2 by Trapp, 2·2 by Loebisch.

b. The acidity of urine is due normally to acid phosphate of soda. The acidity of urine may therefore be reckoned more correctly in terms of phosphoric acid. The proportion of phosphoric acid combined as acid phosphate to the total amount of phosphoric acid present may be taken as a measure of the acidity of urine, and may be estimated in the following way (Huppert):—

The following reagents are necessary:—

i. The reagents necessary to estimate the amount of total phosphoric acid (*see* p. 802).

ii. Standard caustic soda solution containing 10 grammes of caustic soda in a litre of distilled water: 1 c.c. = 0·00591 gramme of phosphoric acid (P_2O_5).

iii. Standard sulphuric acid solution. This contains 12·25 grammes of sulphuric acid (H_2SO_4) in solution. It may be obtained by taking 7·5 c.c. of ordinary sulphuric acid and diluting it to a litre. It must be still a little further diluted until a known volume of it exactly neutralises the same volume of the soda solution. The amount of extra dilution which is necessary is ascertained by titration.

iv. Saturated solution of barium chloride.

v. Neutral litmus solution.

Method.—The total phosphoric acid is first determined with uranium nitrate (*see* p. 802); 200 c.c. of urine are then taken, and rendered strongly alkaline with the soda solution from a burette, the quantity used being noted Call it α. Chloride of barium is then added till no more precipitate occurs. The liquid is filtered. The filtrate is coloured with neutral litmus solution. It is then rendered neutral with the sulphuric-acid solution dropped into it from a burette, the quantity used being noted. Call it β.

Calculation.—When the urine has been rendered alkaline in the way described the unsaturated phosphoric acid (i.e. that combined as acid phosphate) combines with soda, so that phosphate of soda is formed. The barium chloride gives a precipitate of barium phosphate. After the removal of this by filtration the alkalinity of the fluid is determined by the sulphuric-acid solution. The difference in the amounts of acid and alkaline fluids used will correspond to the amount of unsaturated phosphoric acid; from this the quantity of acid combined as acid phosphate can be calculated by the following formula:—

x = quantity of phosphoric acid combined as acid phosphate;

a = quantity in grammes of phosphoric acid which is unsaturated in the urine.

$= (\alpha - \beta) \times {\cdot}0059$

b = total quantity of phosphoric acid in urine (found by the uranium nitrate method);

$x = 2(a - \frac{b}{3})$;

that is, one has to subtract a third of the total phosphoric acid from the amount of unsaturated phosphoric acid and multiply the difference by 2.

The acid phosphate contains in its molecule two parts of phosphoric acid, while in the total phosphates there are three per molecule. One, therefore, finds the relation of the acid phosphate to the total phosphates in molecules by dividing the quantity of phosphoric acid combined as acid phosphate by 2, and that contained in the total phosphates by 3.

Example.—200 c.c. of urine taken.

Total phosphoric acid (b) = 0·2 gramme.

Another 200 c.c. taken. 30 c.c. (α) of soda solution added.

After filtering off the barium phosphate it was found that 9·7 c.c. (β) of the sulphuric acid solution were necessary to neutralise the filtrate.

$$\alpha - \beta = 30 - 9{\cdot}7 = 20{\cdot}3 \text{ c.c.}$$

Each c.c. corresponds to 0·00591 gramme of phosphoric acid.

$$a = (\alpha - \beta) \times 0{\cdot}00591 = 20{\cdot}3 \times 0{\cdot}00591 = 0{\cdot}12 \text{ gr.};$$

$$x = 2\left(a - \frac{b}{3}\right) = 2\left(0{\cdot}12 - \frac{0{\cdot}2}{3}\right) = 0{\cdot}108 \text{ gramme.}$$

The proportion of total phosphate to acid phosphates in molecules

$$= \frac{0{\cdot}2}{3} : \frac{0{\cdot}108}{2} = 66 : 54.$$

ESTIMATION OF CHLORIDES

The chlorides in the urine consist of those of sodium and potassium, the latter only in small quantities.

The method adopted for the determination of the total chlorides consists in their precipitation by a standard solution of silver nitrate or mercuric nitrate.

a. **Mohr's method.**—Precipitation by silver nitrate.

The following solutions must be prepared :—

i. Standard silver nitrate solution. Dissolve 29·075 grammes of fused silver nitrate in a litre (1000 c.c.) of distilled water; 1 c.c. = 0·01 gramme of sodium chloride.

ii. Saturated solution of neutral potassium chromate.

Analysis.—Take 10 c.c. of urine; dilute with 100 c.c. of distilled water.

Add to this a few drops of the potassium chromate solution.

Drop into this mixture from a burette the standard silver nitrate solution: the chlorine combines with the silver to form silver chloride, a white precipitate. When all the chlorides are so precipitated, silver chromate (red in colour) goes down, but not while any chloride remains in solution. The silver nitrate must therefore be added until the precipitate has a pink tinge.

Read off the quantity of standard solution used, and calculate therefrom the quantity of sodium chloride in the 10 c.c. of urine taken, and thence the percentage.

Sources of error and corrections.—A high-coloured urine may give rise to difficulty in seeing the pink tinge of the chromate of silver: this is overcome by diluting the urine more than stated in the preceding paragraph.

1 c.c. should always be subtracted from the total number of c.c. of the silver nitrate solution used, as the urine contains small quantities of certain compounds more easily precipitable than the chromate.

b. To obviate such sources of error the following modifications of the test, as described by Sutton,[1] is used : 10 c.c. of urine are measured into a thin porcelain capsule and 1 gramme of pure ammonium nitrate added; the whole is then evaporated to dryness, and gradually heated over a small spirit lamp to low redness till all vapours are dissipated and the residue becomes white. It is then dissolved in a small quantity of water, and the carbonates produced by the combustion of the organic matter neutralised by dilute acetic acid; a few grains of pure calcium carbonate to remove all free acid are then added, and one or two drops of potassium chromate. The mixture is then titrated with decinormal silver solution (16·966 gr. of silver nitrate per litre) until the end reaction, a pink colour, appears. Each c.c. of silver solution represents 0·005837 gr. of salt; consequently, if 12·5 c.c. have been used, the weight of salt in the 10 c.c. of urine is 0·07296 gr., or 0·7296 per cent. If 5·9 c.c. of urine are taken for titration, the number of c.c. of silver solution used will represent the number of parts of salt per 1000 parts of urine.

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[1] *Volumetric Analysis*, p. 309.

Pribram[1] uses potassium permanganate at a boiling temperature to destroy organic matter instead of ammonium nitrate. Other methods for estimating chlorides are those of Volhard,[2] Habel and Fernholz,[3] Arnold,[4] and Zuelzer,[5] but none are so good when applied to urine as Mohr's.

c. **Liebig's method.**—Precipitation by mercuric nitrate.

The following solutions must be first prepared:—

i. Standard mercuric nitrate solution:—Dissolve 20 grammes of pure mercury in boiling nitric acid; then dilute to nearly a litre. To dilute this to the right strength, preliminary experiments must be performed with a standard solution of pure sodium chloride, 20 grammes to the litre. Take 10 c.c. of the standard sodium chloride solution, add to this 2 c.c. of a 4 per cent. solution of urea, and 5 c.c. of a saturated solution of sodium sulphate. Into this mixture allow the mercuric nitrate solution to flow from a burette, stirring the mixture the while. A precipitate forms, which redissolves on stirring; add the mercuric nitrate solution till a permanent precipitate (not an opalescence) forms; the reaction is then complete. The strength of the mercurial solution is thus determined, and it is then diluted so that 20 c.c. = 0·2 gramme of sodium chloride = 10 c.c. of the standard sodium chloride solution: 1 c.c. therefore corresponds to 0·01 gramme of sodium chloride, or 0·006059 gramme of chlorine.

ii. Baryta mixture.—This is made by adding two volumes of barium hydrate solution to one of barium nitrate solution, both saturated in the cold.

iii. Dilute nitric acid (1 in 20).

Analysis.—Take 40 c.c. of urine.

Add 20 c.c. of baryta mixture. Filter off the precipitate which forms, which consists of sulphate and phosphate of barium.

Take 15 c.c. of the filtrate: this corresponds to 10 c.c. of the original urine.

Render this slightly acid with dilute nitric acid.

Run in the standard mercuric nitrate solution from a burette, stirring the mixture well until a permanent precipitate appears.

Read off the number of c.c. used; multiply by 0·01. This gives the amount of chlorine as sodium chloride contained in 10 c.c. urine.

Explanation and corrections.—This test depends on the fact that when mercuric nitrate and sodium chloride in solution are mixed, sodium nitrate and mercuric chloride, which are both soluble in water, are formed. It is not till all the chloride in the urine is so decomposed that mercuric nitrate begins to combine with the urea present to form a permanent white precipitate. Hence the necessity of estimating the chlorides when using Liebig's method for the determination of urea.

In order to obtain the exact point at which the precipitate becomes a permanent one, the process must be repeated in another specimen. The advantage of this process is its simplicity; its disadvantage is that the end point is rather obscure.

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[1] *Zeit. anal. Chem.* ix. 428.

[2] See *Sutton's Vol. Anal.* p. 810.

[3] *Pflüger's Archiv*, xxiii. 85; xxiv. 2.

[4] *Ibid.* xxxv. 541.

[5] *Ber. deutsch. chem. Ges.* xviii. 38

If the urine used is albuminous the albumin must be first removed by boiling, after the addition of a few drops of acetic acid, and filtering off the precipitated albumin.

ESTIMATION OF THE PHOSPHATES

The phosphoric acid in the urine is combined with soda, potash, lime, and magnesia.

a. **Estimation of the total phosphates.**

For this purpose the following reagents are necessary :—

i. A standard solution of uranic nitrate. The uranic nitrate solution contains 35·5 grammes in a litre of water; 1 c.c. corresponds to 0·005 gramme of phosphoric acid (P_2O_5).

ii. Acid solution of sodium acetate. Dissolve 100 grammes of sodic acetate in 900 c.c. of water; add to this 100 c.c. of glacial acetic acid.

iii. Solution of potassium ferrocyanide.

Method.—Take 50 c.c. of urine. Add 5 c.c. of the acid solution of sodium acetate. Heat the mixture to 80° C.

Run into it while hot the standard uranium nitrate solution from a burette until a drop of the mixture gives a distinct brown colour with a drop of potassium ferrocyanide placed on a porcelain slab. Read off the quantity of solution used and calculate therefrom the percentage amount of phosphoric acid in the urine.

b. **Estimation of the phosphoric acid combined with lime and magnesia** (alkaline earths).

Take 200 c.c. urine. Render it alkaline with ammonia. Lay the mixture aside for twelve hours. Collect the precipitated earthy phosphates on a filter; wash with dilute ammonia (1 in 3). Wash the precipitate off the filter with water acidified by a few drops of acetic acid. Dissolve with the aid of heat, adding a little more acetic acid if necessary. Add 5 c.c. of the acid solution of sodium acetate. Bring the volume up to 50 c.c., and estimate the phosphates in this volumetrically by the standard uranium nitrate, as before. Subtract the phosphoric acid combined with the alkaline earths thus obtained from the total quantity of phosphoric acid, and the difference is the amount of acid combined with the alkalis soda and potash.

c. Instead of uranium nitrate a standard solution of uranium acetate may be used. The directions for the making of these standard solutions will be found in 'Sutton's Volumetric Analysis.' As a rule, it is less troublesome, and not much more expensive, to purchase standard solutions ready made.

ESTIMATION OF THE SULPHATES

The sulphates in the urine are of two kinds: the pre-formed sulphates, viz. those of soda and potash, and the combined or ethereal sulphates.

a. For the determination of the total amount of sulphuric acid (SO_3) (i.e. preformed and combined sulphuric acid together) in the urine one of two methods is adopted :—

1. Volumetric method. 2. Gravimetric method.

1. **Volumetric determination.**—This process consists in adding to a given volume of the urine a standard solution of chloride of barium so long as a precipitate of barium sulphate is formed.

The following solutions are necessary:—

i. Standard barium chloride solution: 30·5 grammes of crystallised chloride of barium in a litre of distilled water; 1 c.c. of this solution corresponds to 0·01 gramme of sulphuric acid (SO_3).

ii. Solution of sulphate of potash: 20 per cent.

iii. Pure hydrochloric acid.

Method.—100 c.c. of urine are taken in a flask. This is rendered acid by 5 c.c. of hydrochloric acid, and boiled. The combined sulphates are thus converted into ordinary sulphates, and give a precipitate like them with barium chloride. The chloride of barium solution is allowed to drop into this mixture as long as any precipitate occurs, the mixture being heated before every addition of barium chloride to it. After adding 5 to 8 c.c. of the standard solution, allow the precipitate to settle; pipette off a few drops of the clear, supernatant fluid into a watch-glass; add to it a few drops of the standard barium nitrate solution. If any precipitate occurs, return the whole to the flask and add more barium chloride; again allow the precipitate to settle, and test as before; go on in this way until no more barium sulphate is formed on the addition of barium chloride.

Excess of barium chloride must also be avoided; when only a trace of excess is present a drop of the clear fluid removed from the flask gives a cloudiness with a drop of the potassium sulphate solution placed on a glass plate over a black ground. If more than a cloudiness appears, too large a quantity of barium chloride has been added, and the operation must be repeated. From the quantity of barium chloride solution used, the percentage of sulphuric acid in the urine is calculated.

2. **Gravimetric determination** (i.e. by weight).—This method consists in weighing the precipitate of barium sulphate obtained by adding barium chloride to a known volume of urine; 100 parts of sulphate of barium correspond with 34·33 parts of sulphuric acid (SO_3).

Method (Salkowski).—100 c.c. of urine are taken in a beaker. This is acidified with 5 c.c. of hydrochloric acid as before.

Chloride of barium is added till no more precipitate occurs.

The precipitate is collected on a small filter of known ash, and washed with hot distilled water till no more barium chloride occurs in the filtrate, i.e. until the filtrate remains clear after the addition of a few drops of hydric sulphate. Then wash with hot alcohol, and afterwards with ether. Remove the filter, and place it with its contents in a platinum crucible. Heat to redness. Cool over sulphuric acid in an exsiccator; weigh, and deduct the weight of the crucible and filter ash; the remainder is the weight of barium sulphate formed.

Error.—When the experiment is carried out as above there is a slight error from the formation of a small quantity of sulphide of barium. This may be corrected as follows: After the platinum crucible has become cool add a few drops of pure sulphuric acid (H_2SO_4). The sulphide is converted into sulphate. Heat again to redness to drive off excess of sulphuric acid.

b. The following is Salkowski's[1] method of estimating the **combined sulphuric acid**; that is, the amount of SO_3 in ethereal sulphates:—100 c.c. of urine is mixed with 100 c.c. of alkaline barium chloride solution, which is a mixture of two volumes of solution of barium hydrate with one of barium chloride, both saturated in the cold. The mixture is stirred, and after a few minutes filtered; 100 c.c. of the filtrate (=50 c.c. of urine) are acidified with 10 c.c. of hydrochloric acid, boiled, kept at 100° C. on the water-bath for an hour, and then allowed to stand till the precipitate has completely settled; if possible, it should be left in this way for twenty-four hours. The further treatment of this precipitate (=combined sulphates) is then carried out as in the last case.

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[1] *Zeit. physiol. Chem.* x. 346. This method is a modification or Baumann's original method, *Ibid.* i. 71.

Calculation.—233 parts of barium sulphate correspond to 98 parts of H_2SO_4 = 80 parts of SO_3 of 32 parts of S. To calculate the H_2SO_4, multiply the weight of barium sulphate by $\frac{98}{233} = 0{\cdot}4206$; to calculate the SO_3 multiply by $\frac{80}{233} = 0{\cdot}3433$; to calculate the S multiply by $\frac{32}{233} = 0{\cdot}13734$. This method of calculation applies to the gravimetric estimation both of total sulphates and of combined sulphates.

c. To obtain the amount of pre-formed sulphuric acid subtract the amount of combined SO_3 from the total amount of SO_3. The difference is the pre-formed SO_3.

Example: 100 c.c. of urine gave 0·5 gramme of total barium sulphate. This multiplied by $\frac{80}{233} = 0{\cdot}171$ gr. = total SO_3. Another 100 c.c. of the same urine gave 0·05 gr. of barium sulphate from ethereal sulphates; this multiplied by $\frac{80}{233} = 0{\cdot}017$ gr. of combined SO_3. Total SO_3 − combined $SO_3 = 0{\cdot}171 - 0{\cdot}017 = 0{\cdot}154$ gr. of pre-formed SO_3.

ESTIMATION OF THE CARBONIC ACID

Carbonic acid occurs in the urine both in the free state and also combined with alkaline metals to form carbonates.

a. *Estimation of the free carbonic acid* (Marchand).—100 c.c. of urine are put into a glass flask closely fitted with a doubly perforated cork. Through one opening a tube is passed which dips into the urine, and at the other end is connected with a tube containing pieces of quicklime. Through the other opening in the cork one arm of a doubly bent tube is passed; this does not dip into the urine; the other arm is introduced into an empty flask through a well-fitting cork. This flask is connected by a similar tube with a second flask filled with clear baryta-water, and this with a third and fourth filled with baryta-water.

The urine is then heated to 100° C. over a water-bath; any portions of it that boil over go into the empty flask. The carbonic acid comes off and forms a white precipitate of barium carbonate in the flasks filled with baryta-water. Air is then drawn through the apparatus; any carbonic acid in the atmosphere being removed by the quicklime. The carbonate of baryta formed is collected on a filter, washed with distilled water, dissolved in hydrochloric acid, precipitated again by sulphuric acid, and weighed as barium sulphate. From the quantity so obtained the amount of carbonic acid in the urine can be calculated; 196·65 parts of barium carbonate correspond to 232·62 parts of barium sulphate, and 44 parts of carbonic acid.

b. *The total carbonic acid* is similarly estimated after strongly acidifying the urine with hydrochloric or phosphoric acid.

The combined carbonic acid is the difference between the total and the free carbonic acid.

ESTIMATION OF THE POTASH AND SODA

a. *Of the potash and soda together.*—30 c.c. of urine are mixed with 30 c.c. of baryta mixture (two volumes of barium hydrate solution to one of barium nitrate solution, both saturated in the cold). The precipitate which forms is filtered off, 40 c.c. of the filtrate (corresponding to 20 c.c. of urine) are evaporated to dryness in a platinum capsule in a water-bath. The residue is incinerated, heating gently at first till nearly all the carbon is burnt.

To the residue boiling water is added and then carbonate of ammonia as long as a precipitate is thrown down. The precipitate is filtered off, washed, and the filtrate and washings, acidified with hydrochloric acid, evaporated to dryness in a platinum crucible of known weight. The dried residue is gently heated to drive off salts of ammonia, cooled over sulphuric acid in an exsiccator, and weighed. The weight, *minus* that of the crucible, is that of the total sodium and potassium combined with chlorine.

b. *Of the potash alone.*—Dissolve the two chlorides obtained as above in a little water. Add excess of platinic chloride, and evaporate almost to dryness in a water-bath. Treat the residue with 80 per cent. alcohol, and allow it to stand some hours. The sodio-chloride of platinum alone dissolves. Collect the undissolved potassio-chloride of platinum on a filter of known weight, wash with 80 per cent. alcohol, dry at 100° C., and weigh; 100 parts of potassio-chloride of platinum correspond to 30·51 parts of chloride of potassium. From this the percentage of chloride of potassium can be calculated.

The combined weight of the two chlorides, *minus* that of the potassium chloride, gives the weight of the sodium chloride.

From this the amount of potash (K_2O) and soda (Na_2O) can be calculated, one part of chloride of potassium corresponding to 0·6317 of potassic oxide (K_2O), and one part of sodic chloride corresponding to 0·5302 of sodic oxide (Na_2O).

ESTIMATION OF LIME

Lime may be estimated either by a volumetric method or by a gravimetric method.

a. **Volumetric method.**—The lime is precipitated by oxalate of ammonia as oxalate of lime; by heat this is converted into caustic lime and carbonate of lime, the amount of which is ascertained by a standard acid solution.

The following reagents are necessary:—

i. Standard hydrochloric acid solution.—60 c.c. of hydrochloric acid are diluted nearly to a litre; it is then placed in a burette, and diluted until it is found that 1 c.c. of it just neutralises a solution of caustic soda containing 20 grammes to the litre; 1 c.c. of this acid solution corresponds to 0 014 gramme of lime (CaO).

b. Standard caustic soda solution; 20 grammes to the litre.

ii. Ammonia solution.

iii. Oxalate of ammonia solution.

iv. Acetic acid.

v. Neutral litmus solution.

Analysis.—Take 200 c.c. of urine.

Add ammonia till a large precipitate occurs. Collect and redissolve the precipitate carefully by acetic acid, adding only a few drops of acid in excess. To this add oxalate of ammonia, and allow it to stand six or eight hours till a precipitate of oxalate of lime settles.

Syphon off the clear, supernatant fluid, and collect the precipitate on a small filter, and wash with hot water.

N.B.—Preserve the supernatant fluid, filtrate, and washings for the estimation of the magnesia,

Incinerate the filter with the precipitate; lime and carbonate of lime are thus formed.

To this residue add 10 c.c. of the standard acid solution, and heat carefully to expel all the carbonic acid. By this means all the calcium present is combined as chloride. Colour the liquid with neutral litmus solution, and estimate the acidity by the standard soda solution.

Subtract the number of cubic centimetres of soda solution used from the 10 c.c. of the acid solution employed. The remainder is the number of c.c. of acid solution employed to saturate the lime present.

Then calculate from this the percentage of lime.

b. **Determination by weight.**—One proceeds as above till a precipitate of oxalate of lime is obtained from 200 c.c. of urine. This is collected on a filter of known ash, well washed, and incinerated till the weight becomes constant in a platinum crucible of known weight. Cool over sulphuric acid in an exsiccator, and weigh. Subtract the weights of crucible and filter ash, and the remainder gives the amount of lime (CaO) present in 200 c.c. of urine.

ESTIMATION OF MAGNESIA

This is best determined by weight.

The fluid separated from the oxalate of lime in the preceding experiment is treated with ammonia till alkaline. In this way all the magnesia is thrown down as ammonio-phosphate of magnesia. Allow some hours for this to settle; collect on a filter of known ash, and wash with dilute ammonia (1 in 4).

Incinerate in a platinum crucible till white; cool over sulphuric acid, and weigh. The incineration is hindered by the presence of uric acid, but can be hastened by adding a small piece of nitrate of ammonia moistened with distilled water to the precipitate.

Heat converts the ammonio-phosphate into pyrophosphate of magnesia, 100 parts of which correspond to 36·03 of magnesia (MgO).

ESTIMATION OF AMMONIA (SCHLOESING)

The following solutions are necessary:—

i. Standard sulphuric acid.—This contains 49 grammes of sulphuric acid (H_2SO_4) in the litre. It may be made by adding about 30 c.c. of concentrated sulphuric acid to a litre of water, and then by titration this is further diluted till one volume of it exactly neutralises one volume of a standard solution of caustic soda which contains 40 grammes to the litre; 1 c.c. of the acid solution corresponds to 0·017 of ammonia (NH_3).

ii. Standard soda solution containing 10 grammes to the litre.

iii. Milk of lime.

iv. Neutral litmus solution.

Method.—20 c.c. of urine freed from mucus by filtration are placed in a beaker. A triangle made of glass rod is laid upon it, and upon the triangle is placed a shallow vessel containing 10 c.c. of the standard sulphuric acid solution. The two are placed on a glass plate, and covered with a bell-jar, of which the edges are well greased. Raise the bell-jar, add quickly to the urine 10 c.c. of milk of lime, and immediately replace the bell-jar.

In forty-eight hours the whole of the ammonia is driven off from the urine and absorbed by the sulphuric acid.

The sulphuric acid is then coloured with neutral litmus solution. Its acidity is then measured with the soda solution, four volumes of which corresponds to one of the acid solution.

Divide the number of c.c. of soda solution used by 4; subtract this from the 10 c.c. of acid solution used. The remainder is the number of c.c. of acid employed to saturate the ammonia present, each c.c. of acid so used corresponding to 0·017 gramme of ammonia (NH_3).

Control experiment.—Perform a similar experiment with urine to which no milk of lime has been added, and thus estimate the amount of ammonia which has formed in forty-eight hours from the decomposition of urea. Subtract this from the quantity found in the first experiment.

As a rule, however, fresh, healthy urine, if freed from mucus, does not decompose in forty-eight hours.

ESTIMATION OF TOTAL NITROGEN

This is best accomplished by Kjeldahl's method (*see* p. 23).

5 c.c. of urine and 20 c.c. of the mixed acids are measured into a flask of about 300 c.c. capacity, and heated to boiling. The heat is continued till all vapours cease to come off, and the fluid possesses a clear yellow tint. Twenty-five to thirty minutes generally suffice. The flask is then allowed to cool, diluted, and the liquid distilled with caustic soda and zinc into a known volume of standard acid, as already described. The loss of acidity, ascertained by titration with standard alkali, is a measure of the amount of ammonia given off by distillation, and from this the amount of nitrogen is calculated.

ESTIMATION OF URIC ACID

a. An approximate, and, for most clinical purposes, sufficiently accurate process is the following (Heintz' method):—

Take 100 c.c. of urine. Add to this 5 c.c. of hydrochloric acid. Lay the mixture aside for twenty-four hours. Collect the crystals on a weighed filter paper, wash with dilute hydrochloric acid, dry at 100° C., and weigh. The increase in weight will give the percentage of uric acid.

b. In some cases, however, urine containing uric acid gives no precipitate in this way, and many attempts have been made to find a thoroughly trustworthy method. Haycraft[1] invented a method based on the fact that uric acid combines with silver as silver urate; the silver urate is collected, dissolved in nitric acid, and the silver estimated volumetrically by Volhard's method.[2] From the amount of silver found the amount of uric acid is calculated. Herrmann[3] obtained good results by this method, and Czapek[4] slightly modifying the process found a large error. Salkowski[5] also regards the process as of little value, as the composition of the silver urate is not constant; in this opinion he is supported by Gossage.[1] As, therefore, there is a doubt as to the applicability of Haycraft's method to urine, I do not propose to give an account of it here.

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[1] *Brit. Med. Journ.* December 1885.

[2] *Liebig's Annalen*, cxc. 1.

[3] *Zeit. physiol. Chem.* xii. 496.

[4] *Ibid.* p. 502.

[5] *Ibid.* xiv. 31.

c. *Fokker's method*[2] (modified by Salkowski[3]) is as follows:—200 c.c. of urine are made strongly alkaline with sodium carbonate, and after an hour 20 c.c. of a concentrated solution of ammonium chloride are added. The mixture is allowed to stand at a low temperature for forty-eight hours, the precipitate which forms collected on a weighed filter, and washed. The filter is filled with dilute hydrochloric acid (1 in 10), and the filtrate collected; this operation is repeated till all the acid urate on the filter is dissolved. The filtrates are mixed, allowed to stand for six hours, and the uric acid which then separates is collected on the same filter, washed twice with water, then with alcohol, till all acid reaction disappears, dried at 110° C., and weighed. To the weight obtained add 0·03 gramme, and subtract the weight of the filter; the remainder is the weight of uric acid in 200 c.c. of urine.

d. *Camerer's method.*[4]—Camerer has subjected to a most thorough examination all the various hitherto proposed methods for the estimation of uric acid, finds none thoroughly satisfactory, and proposes the following new one, which appears to be the best up to the present:—The twenty-four hours' urine is mixed with a measured amount of dilute solution of caustic soda (500 c.c. of water containing from 0·4 to 1 gramme of soda was found to be the best proportion). This precipitates the earthy phosphates, which are then filtered off. The mixture is then diluted with water till its specific gravity is 1010 to 1011; if, however, the urine is rich in uric acid, the dilution must be greater; if poor, less. To 300 c.c. of this diluted urine are added 50 c.c. of Salkowski's[5] magnesia mixture (1 part of crystallised magnesium sulphate, 2 parts of ammonium chloride, 4 parts of ammonia solution of specific gravity 0·924, and 8 parts of water) to precipitate the rest of the phosphates; filter this off. The first 30 c.c. of the filtrate are used to wash out the measuring glass, the next 175 c.c. (= 150 of diluted urine) are used for analysis; place this in a beaker containing 0·5 gramme of finely divided calcium carbonate; then about 5 c.c. of a 3 per cent. solution of silver nitrate; the precipitate which forms is allowed to settle; the supernatant liquid is tested for silver; if it contains none, more of the silver nitrate solution must be added. When the supernatant solution gives evidence of excess of silver, proceed with the analysis. The precipitate is collected, the calcium carbonate preventing it going through the filter; it is well washed with water till quite free from silver and from chlorides, and then dried over sulphuric acid in an exsiccator. An estimation of the nitrogen in this precipitate is then made by Kjeldahl's method (*see* p. 23); each part of nitrogen found corresponds to 3 parts of uric acid. This gives the amount of uric acid in 150 c.c. of the diluted urine. The amount of dilution being known, the percentage of uric acid in the urine is easily ascertained, and from this the quantity in the day's urine is then found.

Camerer[6] points out that there are two possible objections to this method: (1) A loss due to imperfect filtration; this can be easily obviated by the use of Schleicher and Schull's papers. (2) Xanthine compounds are reckoned as uric acid. This latter objection is a serious one. Camerer therefore compared his method with that of Ludwig, in which pure uric acid is separated out, silver being got rid of by the use of hydrogen sulphide. Ludwig's method is a tedious and laborious one. The mean difference between Camerer's and Ludwig's method was found to be 11 per cent. Camerer suggests that, knowing this, the real percentage of uric acid can be found by calculation; and the results thus obtained are remarkably accurate. To give an illustration: the silver precipitate of 150 c.c. of urine yielded 14·39 milligr. of nitrogen or 9·6 milligr. per 100 c.c. 9·6 × 3 = 28·8 = percentage of uric acid by Camerer's method. 28·8 – (28·8 × 0·11) = 25·6 = percentage of true uric acid by calculation. This is very close to 26·00, which was the percentage found by Ludwig's method.

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[1] *Proc. Roy. Soc.* xliv. 284.

[2] *Pflüger's Archiv*, x. 153.

[3] *Virchow's Archiv*, lxviii. 401. *See* also Pott, *Pflüger's Arch.* xlv. 389.

[4] *Zeit. Biol.* xxvi. 84.

[5] *Pflüger's Archiv*, v. 319.

[6] *Zeit. Biol.* xxvii. 113.

ESTIMATION OF HIPPURIC ACID

The method of estimation consists in the preparation of pure hippuric acid from a known quantity of urine, and weighing it.

Method (Bunge and Schmiedeberg).—200 c.c. of urine are taken. This is rendered alkaline with sodic carbonate and evaporated to dryness. The residue is extracted with cold alcohol, and the extract distilled until all the alcohol has passed off. The remaining watery fluid is rendered acid with hydrochloric acid, and shaken at least five times with fresh portions of acetic ether. The acetic ether is washed by shaking with water, and evaporated at a moderate temperature. The residue consists of hippuric acid, benzoic acid, and fat. It is extracted with petroleum ether (light petroleum); the hippuric acid alone remains undissolved. The residue of hippuric acid is dissolved in a little warm water, the solution passed through animal charcoal. It is then evaporated to dryness on a weighed capsule at a temperature of 50° to 60° C. The crystals consist of hippuric acid. Weigh; the weight, *minus* that of the capsule, is the amount of hippuric acid in 200 c.c. of urine.

ESTIMATION OF OXALIC ACID

This is a gravimetric process, the oxalic acid being weighed as oxalate of lime.

Method (Neubauer).—400 to 600 c.c. of urine are taken. Solution of chloride of calcium is added. Excess of ammonia is added, and the precipitate which forms, dissolved in acetic acid, excess being avoided. Oxalate of lime, however, remains undissolved. Let this settle for twenty-four hours. Some small amount of uric acid is generally deposited also. Collect the precipitate on a small filter; wash with water; then place the filter together with the precipitate in hydrochloric acid, and warm; the uric acid is not dissolved, the oxalate of lime is. Filter off the undissolved uric acid, wash with dilute hydrochloric acid, and add the washings to the filtrate. Neutralise this with dilute ammonia; crystals of oxalate of lime separate out, and are collected on a filter of known weight, weighed, and the quantity of oxalic acid calculated therefrom, 100 parts of calcic oxalate corresponding to 70·31 parts of oxalic acid ($C_2H_2O_4$).

Or after the crystals of oxalate of lime are obtained, the process may be modified as follows (Czapek):—Collect the crystals on a filter of known ash. Wash with dilute acetic acid, and then with distilled water. Incinerate precipitate and filter paper in a platinum crucible until no more weight is lost. The oxalate is first changed into carbonate of lime (carbonic oxide being given off), and then into lime or oxide of calcium, carbonic acid being given off. $CaC_2O_4 = CaO + CO_2 + CO$. About twenty minutes is generally sufficient for the decomposition. From the final weight of the contents of the crucible the amount of filter ash is deducted; the remainder is that of the lime formed from the oxalate of calcium. This, multiplied by 1·6071, gives the amount of oxalic acid in the quantity of urine used.

ESTIMATION OF UREA

If albumin is present it must be first separated by boiling after acidulation with acetic acid if necessary, and filtering off the flakes of coagulated proteid. The two chief methods of estimating urea are:—

a. The mercuric nitrate, or Liebig's method.

b. The hypobromite, or Hüfner's method.

a. **Liebig's method.**—The combination between urea and mercuric oxide has been alluded to in the account just given of Liebig's method of estimating chlorides; this combination $[(CON_2H_4)_2Hg(NO_3)_2 + 3HgO]$ forms a white precipitate, insoluble in water and weak alkaline solutions. It is, therefore, necessary to prepare a standard solution of mercury, and to have an indicator by which to detect the point when all the urea has entered into combination with the mercury, and the latter slightly predominates. This indicator is sodium carbonate, which gives a yellow colour with the excess of mercury, owing to the formation of hydrated mercuric oxide.

Theoretically, 100 parts of urea should require 720 parts of mercuric oxide; but, practically, 772 of the latter are necessary to remove all the urea, and at the same time show the yellow colour with alkali; consequently the solution of mercuric nitrate must be of empirical strength in order to give accurate results.

The following solutions must be prepared:—

i. Standard mercuric nitrate solution. Dissolve 77·2 grammes of red oxide of mercury (weighed after it has been dried over a water-bath) or 71·5 gr. of the metal itself, in dilute nitric acid. Expel excess of acid by evaporating the liquid to a syrupy consistence. Make up to 1000 c.c. with distilled water, adding the water gradually. This solution is of such a strength that 19 c.c. will precipitate 10 c.c. of a 2 per cent. urea solution. Add 52·6 c.c. of water to the litre of the mercuric nitrate solution, and shake well; then 20 c.c. (instead of 19) = 10 c.c. 2 per cent. urea solution, i.e. 1 c.c. = ·01 urea.

ii. Baryta mixture.—This is a mixture of two volumes of solution of barium hydrate with one of solution of barium nitrate, both saturated in the cold.

Analysis.—Take 40 c.c. urine. Add to this 20 c.c. baryta mixture and filter off the precipitate of baryta salts (phosphates and sulphates). Take 15 c.c. of the filtrate (this corresponds to 10 c.c. of urine) in a beaker. Run into it the mercuric nitrate solution from a burette, until on mixing a drop of the mixture with a drop of a saturated solution of sodium carbonate on a white tile a pale lemon colour appears. Then read the amount used from the burette, and calculate thence the percentage of urea.

Corrections.—This method only approaches accuracy when the quantity of urea present is about 2 per cent., which is about the normal percentage of urea in urine. The chlorine in the urine must also be estimated, and the quantity of urea indicated reduced by the subtraction of 1 gramme of urea for every 1·3 gramme of sodium chloride found. If the urine contains less than 2 per cent. of urea, 0·1 c.c. of mercuric nitrate solution must be deducted for every 4 c.c. used; if more than 2 per cent. of urea, a second titration must be performed with the urine diluted with half as much water as has been needed of the mercurial solution above 20 c.c. Suppose, then, 28 c.c. have been used in the first titration, the excess is 8 c.c.; therefore 4 c.c. of water must be added to the urine before the second titration is made. When ammonium carbonate is present, first estimate the urea in one portion of urine, and the ammonia by titration with normal sulphuric acid in another; 0·017 gramme of ammonia = 0·030 of urea.

The equivalent of ammonia in terms of urea must be added to the urea found in the first portion of urine.

Modifications.—Rautenberg[1] and Pflüger[2] have devised modifications of Liebig's original method. Rautenberg's method consists in maintaining the urea solution neutral throughout by successive additions of calcium carbonate. Pflüger's method is as follows: A 2 per cent. solution of urea is prepared; 10 c.c. of this are placed in a beaker, and 20 c.c. of the mercuric nitrate solution are run into it in a continuous stream; the mixture is then brought under a burette containing normal sodium carbonate, and this is added with constant agitation until a permanent yellow colour appears. The volume so used is noted as that necessary to neutralise the acidity produced by 20 c.c. of the mercurial solution in the presence of urea. A plate of glass is then laid on black cloth, and some drops of a thick mixture of sodium bicarbonate (free from carbonate) and water placed upon it at convenient distances. The mercurial solution is added to the urine in such volume as is judged appropriate, and from time to time a drop of the white mixture is placed beside the bicarbonate, so as to touch but not mix completely. A point is at last reached when the white gives place to yellow; both drops are then rubbed quickly together with a glass rod, and the colour disappears; further addition of mercury is then made to the urine till a drop rubbed with the bicarbonate remains permanently yellow. Now is the time to neutralise by the addition of the normal sodium carbonate to near the volume found necessary in the preliminary experiment. If this is quickly done a few tenths of a c.c. of mercuric nitrate will be found sufficient to complete the reaction. If, however, much time has been lost, it may happen that, notwithstanding the mixture is distinctly acid, it gives, even after the addition of sodium carbonate, a permanent yellow, although no more mercuric nitrate be added. The analysis must be under those circumstances repeated, taking the first titration as a guide to the quantities which are necessary. Pflüger's correction for concentration of urea is different from Liebig's, and is as follows:—

V^1 = volume of urea solution + volume of sodium carbonate solution + volume of any other fluid free from urea which may be added.

V^2 = volume of mercuric nitrate solution used.

$C = \text{correction} = -(V^1 - V^2) \times 0{\cdot}08$.

This formula holds good for cases where the total mixture is less than three times the volume of mercuric nitrate solution used; with more concentrated solutions the formula gives results too high.

Pflüger and Bleibtreu ('Pflüger's Archiv,' xliv. p. 1) have recently in a series of papers introduced fresh methods of urea analysis of so complex a nature that they are quite unsuitable for ordinary clinical work.

b. **The hypobromite method.**—This is a far more accurate and easier method. The method consists in decomposing urea into water, carbonic acid, and nitrogen by means of an alkaline solution of hypobromite of soda; the carbonic acid combines with the soda, and the nitrogen which is evolved is measured, and the quantity of urea therefrom calculated. There are many kinds of apparatus for performing this operation, but the best yet devised are those of Dupré[3] and Gerrard.[4]

The apparatus and reagents one requires for the determination are as follows:—

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[1] *Ann. Chem. Pharm.* cxxxiii. 55.

[2] *Zeit. anal. Chem.* xix. 375. Pfeiffer (*Zeit. Biol.* xx. 540) has made a careful comparison of the different methods proposed.

[3] Dupré, *Journ. of the Chem. Soc.* May 1877.

[4] *Lancet*, ii. 1884, p. 952.

i. A Dupré's apparatus [1] or a Gerrard's apparatus.[2]
ii. A 5 cubic centimetre pipette.
iii. A strong glass cylinder with a well-fitting glass stopper.
iv. A 40 per cent. solution of caustic soda.
v. Tubes containing 2 and 4 c.c. of bromine.

The two last-named reagents are required for the making of the hypobromite solution, which spoils by keeping (bromate of soda being formed). It should, therefore, be prepared fresh before every determination.

The hypobromite solution is made by introducing 23 c.c. of the soda solution into the glass cylinder, then gently dropping in a tube containing 2 c.c. of bromine. The tube is then broken by shaking the cylinder, which is stoppered; the bromine escapes, and combines with the soda. This method prevents any inconvenience arising from fumes of bromine. The quantity of solution so prepared is sufficient for one estimation. This procedure is, as Dupré points out, one of the most valuable points about his method; the solution can be made with perfect safety by the bedside.

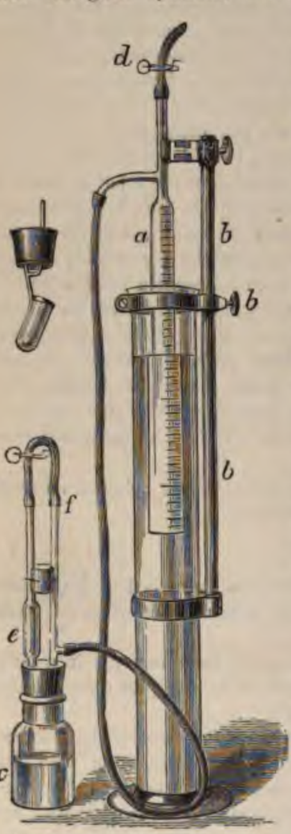

FIG. 100.—Dupré's Urea Apparatus.

Method 1 (Dupré).—Measure 5 c.c. of urine, and introduce it into the test-tube attached to the caoutchouc stopper seen on the upper left-hand side of fig. 100; this will be found simpler to use than the pipette (*e*, *f*) figured below.

Measure 25 c.c. of hypobromite of soda solution, and introduce it into the bottle, *c*.

Close the bottle carefully with the stopper just mentioned, taking care to upset none of the urine in the test-tube attached to it. This stopper is perforated by a glass tube, which is connected by indiarubber tubing to the tube, *e*, by a T-piece. Open the pinch-cock, *d*, and lower the tube, *a*, until the surface of the water with which the outer cylinder is filled is at the zero point of the graduation.

Close the pinch-cock, *d*, and raise *a* to ascertain if the apparatus is air-tight; then lower it again. Tilt *c* so as to upset the urine, and shake well for a minute or so.

Immerse *c* in a large beaker containing water of the same temperature as that in the cylinder. After two or three minutes raise the measuring-tube, *a*, until the surfaces of the liquid inside and outside coincide.

Read off the quantity of nitrogen by means of the graduations on *a* that results from the decomposition of the 5 c.c. of urine. Some of the tubes of Dupré's apparatus are graduated in divisions corresponding to percentages of urea.

The total quantity of urine passed in the twenty-four hours being measured, the total amount of urea excreted in the day can be calculated. If the nitrogen is measured in c.c., 35·4 c.c. of nitrogen corresponds to 0·1 gramme of urea.

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[1] How and Co., Farringdon Street.

[2] Gibbs, Cuxson, and Co., Wednesbur

Reactions and corrections.—The reaction by which urea is decomposed in this proceeding may be denoted by the following formula:—

$$CON_2H_4 + 3NaBrO = CO_2 + N_2 + 2H_2O + 3NaBr.$$

From 1 gramme of urea 0·46 gramme of nitrogen = 372·7 c.c. are obtained.

In practice, however, it is found that only 354·3 c.c. are obtained,[1] except in diabetic urine, in which the urea yields nearly the normal amount of nitrogen. Moreover, urine contains small quantities of creatinine and urates, which yield some of their nitrogen when acted on by sodic hypobromite. When great exactitude is required these must be removed—creatinine by an alcoholic solution of zinc chloride, and the urates by acetate of lead followed by sodic phosphate (Yvon).

5 c.c. of a 2 per cent. solution of urea in urine yield 35·4 c.c. of nitrogen. This quantity is taken as representing 2 per cent. of urea, and serves as a basis for the graduations of the tubes which are marked in percentages.

When very great exactitude is required the quantity of nitrogen must be measured in cubic centimetres, and the volume obtained corrected for temperature, pressure, and tension of aqueous vapour by the formula given on p. 35.

Method 2 (Gerrard).—In the method the hypobromite solution is prepared as before, and introduced into the bottle, *a* (fig. 101). A stout test-tube containing 5 c.c. of urine is carefully lowered by forceps into this.

By means of the short tube, *c*, the long graduated one is filled with water up to the zero mark. *a* is now connected to this latter tube by indiarubber tubing, as in Dupré's apparatus. The urine and hypobromite are mixed by tilting the bottle, *a*; the nitrogen comes off, and is measured in percentages of urea by the graduations on the tube, U. After waiting ten minutes to allow the temperature of the apparatus and the contained gas to reach that of the atmosphere, the water in the two tubes is brought to the same level by lowering the tube, *c*; the reading is then made, and corrected, if necessary, for temperature, pressure, and tension of aqueous vapour as before.

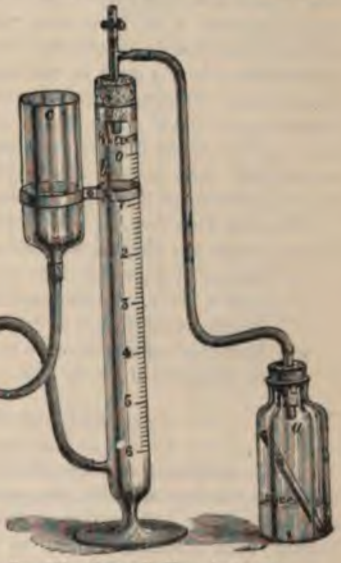

Fig. 101.—Gerrard's Urea Apparatus.

ESTIMATION OF CREATININE

The crystalline compound which creatinine forms with zinc chloride is employed in estimating the quantity of creatinine in urine, 100 parts of the compound corresponding to 62·42 of creatinine.

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[1] The cause of this loss of nitrogen has been investigated by Luther, *Zeit. physiol. hem.* xiii. 500. He finds part is combined as a nitrate, and part in an unknown organic which gives off ammonia when distilled with alkali.

Method.—Take 250 c.c. of urine. Add milk of lime and calcic chloride in excess to precipitate the phosphates. Filter, and evaporate the filtrate to a small bulk; to this add 50 c.c. absolute alcohol, and let the mixture stand for six hours. Then add 10 or 15 drops of an alcoholic solution of zinc chloride; the crystals form, and after two or three days' standing in a dark place may be collected on a weighed filter.

Wash with 90 per cent. alcohol, dry and weigh, and thence calculate the percentage of creatinine.

ESTIMATION OF SUGAR

The quantitative determination of sugar in urine may be made by the different processes already described under the heading Dextrose in Chapter IX. Estimations by the saccharimeter can only be made when the urine is perfectly clear and free from other substances that rotate the plane of polarised light. The fermentation method is so inaccurate that it should now be altogether discarded. Fehling's method is practically the only one now in use; if the urine is albuminous the albumin must be first separated by acidulating with dilute acetic acid, boiling, and filtering. Most diabetic urines are so rich in sugar that it is necessary to dilute them to ten or twenty times their original volume before placing them in the burette; this must, of course, be allowed for in the subsequent calculation. The method of analysis itself will be found described on p. 98, and the composition of Fehling's solution on p. 95.

The following formula will be found useful in estimating the amount of sugar in urine when the English weights and measures are employed:—

x = number of grains of sugar in twenty-four hours.

b = number of c.c. of urine used from burette to decompose 10 c.c. of Fehling's solution (equivalent to 0·05 gramme = 0·77 grain of sugar).

a = number of ounces of urine in twenty-four hours.

28·396 = number of c.c. in 1 oz.

$$x = \frac{a}{b} \times 28{\cdot}396 \times 0{\cdot}77 = \frac{a}{b} \times 21{\cdot}865.$$

Pavy's modification of the above test consists in the addition of ammonia to the copper solution. The composition of Pavy's solution is 34·65 grammes of copper sulphate, 170 gr. of Rochelle salt, 170 gr. of caustic potash dissolved to 1 litre with distilled water; to every 120 c.c. of this mixture 400 c.c. of ammonia (specific gravity 0·88) are added, and diluted to 1 litre with water. 1 c.c. of this = 10 c.c. of Fehling's solution. The resulting solution is a deep blue one, like Fehling's solution. The test is performed as in Fehling's method; the diabetic urine (diluted to a known extent if necessary) is run into the hot Pavy's solution from a burette until all the blue colour disappears; there is, however, no formation of a red or yellow precipitate, as the ammonia holds the reduced oxide in solution; the blue colour simply gradually fades from the solution until when enough sugar is present all blue has disappeared. The disadvantage of this test consists in the fact that the ammonia fumes coming off from the liquid, which must be kept boiling, are so unpleasant that it must be performed in a flask closed with a cork through which two holes are bored; through one of these holes a short piece of glass tube is passed; this is connected to the burette by a piece of india-rubber tubing; through the other hole a long piece of glass tubing is passed through which the ammonia fumes pass out; but these are in great measure condensed in the tube, and return to the flask.

Another modification introduced by Gerrard is the invention of a *percentage glycosometer*.[1] This is designed to save time in calculation, and is of especial service to the clinical observer.

The instrument consists of a pair of burettes (fig. 102) clasped by a pair of swinging arms supported on an upright brass stand; the swinging arrangement allows the burettes to be moved at will, and to be brought over a dish containing Fehling's solution. Fig. 103 shows the burettes on an enlarged scale. They are graduated not in c.c., but in percentages of sugar, for urine which has been diluted to twenty times its volume before placing it in the burettes. The method of graduation is very simple, and will be found fully explained in the article quoted. The narrow burette indicates high, the wide one low percentages, the total range of the two being from 1 to 10 per cent. In performing the analysis both burettes are filled with the diluted urine; this is delivered into the boiling Fehling's solution (10 c.c. diluted with 50 c.c. of water), first from the small and then, if necessary, from the large burette till all blue colour has gone. Read the quantity used in percentages of sugar. Should any urine contain more than 10 per cent. of sugar, let one volume be diluted to 40 with water, proceed as before, and to get the real percentage multiply by 2.

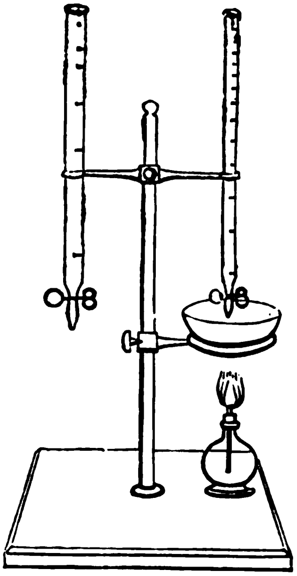

FIG. 102.

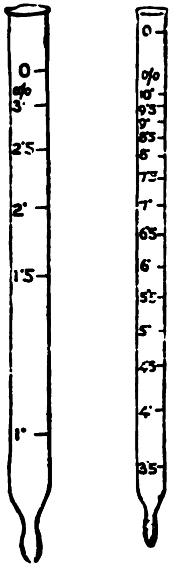

FIG. 103.

Knapp's, Sachsse's, Vogel's, and Johnson's method may also be used for the estimation of sugar in urine (*see* p. 99).

ESTIMATION OF PROTEIDS

a. *For accurate analyses* of total proteids the methods described on pp. 126, 127 should be used; of these that numbered 2 is, in my own experience, the best.

b. **For the estimation** of proteids when albumin and globulin are the only ones present, *Zahor's densimetric process* (p. 127) will be found to give fairly accurate results.

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[1] , vol. i. 1890, p. 15. Made by Gibbs, Cuxson, and Co., Wednesbury.

c. *Clinical methods*, albumin and globulin being estimated together.

The usual clinical method is to boil the urine in a graduated tube. Allow the coagulum to settle and read off the proportion of precipitate to total liquid by the relative space occupied by each.

Esbach completely precipitates the proteid by picric acid (10 grammes of picric acid, 20 gr. citric acid in a litre of water) in a tube which is so graduated that the depth of the deposit at the end of twenty-four hours indicates so many grammes of proteid per litre of urine.

Christensen[1] has proposed a more elaborate but hardly more accurate method. It consists in the use of tannin as the precipitant, and the suspension of the precipitate in the urine by means of mucilage. The mixture is then, after being diluted with water, poured into a vessel of a certain capacity, which is placed over a white surface on which black lines are drawn. The amount of 'emulsified' urine necessary to obscure the lines will be in inverse ratio to the quantity of albumin in the urine; a quantity easily estimated by the employment of a suitably graduated burette. The principle is thus the same as that introduced by Panum for the determination of cream in milk, and can no doubt be made available for clinical work.

d. *Estimation of relative proportion of serum-albumin and serum-globulin* (albumoses and peptones being absent).

For the purposes of accurate analysis, the total proteids are first estimated by the alcohol method in one portion of urine (*see* p. 126); another portion of urine is then neutralised, and the serum-globulin estimated by Hammarsten's magnesium sulphate method (*see* p. 238). The difference between the two gives the amount of serum-albumin.

A useful clinical method is given by Noel Paton.[2] The total proteids are estimated by Esbach's method: 50 c.c. of urine are rendered faintly alkaline with a drop or two of caustic potash, saturated with magnesium sulphate by agitation with excess of the powdered crystals of that salt. The volume of the mixture is measured; in round numbers the volume is 75 c.c., so that 3 c.c. correspond to 2 of urine. Allowing for this subsequently, it is filtered; an Esbach's tube is filled with the filtrate, and the picric acid solution added; the mixture is allowed to stand five days, and then the reading is made. This deducted from the total proteids gives the amount of serum-globulin.

e. *Estimation of other proteids.* Fibrinogen, *see* p. 236. Fibrin, *see* p. 233. Oxy-hæmoglobin and methæmoglobin; an approximate determination may be made with Gower's hæmoglobinometer (p. 283); the tint of oxy-hæmoglobin in the urine (and still more is this the case with methæmoglobin) is, however, different from that of the oxy-hæmoglobin obtained from the circulation. For the estimation of albumoses or peptones only approximate methods can as yet be carried out; the precipitate produced by alcohol or by tannin may be collected and weighed; if albumin and globulin are also present, the urine must be first freed from these by acidulation, boiling, and filtering.

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[1] *Virchow's Archiv*, cxv. 128.

[2] *Edin. Med. Journ.* 1888, p. 522.

ESTIMATION OF FAT

20 to 30 c.c. of urine are evaporated to dryness in a water-bath. The residue is dried at 110° C. This is then extracted with ether for some time. This is poured off, and fresh ether added as long as it takes anything up. The ethereal extracts are then evaporated in a glass tube of known weight, at a low temperature, and the residue is calculated as fat.

When pus occurs in the urine or in chyluria the ether takes up not only fat, but also lecithin and cholesterin. The relative amounts of these constituents may be estimated as described on p. 533.

ESTIMATION OF PHENOL (CARBOLIC ACID)[1]

100 c.c. of urine are concentrated on a water-bath to a fifth part of that volume.

Concentrated sulphuric acid is then added in such a quantity that the liquid contains 5 per cent. of sulphuric acid.

Distil till the distillate is not rendered cloudy by the addition of bromine water. The distillate is filtered if necessary, and coloured a permanent light yellow with bromine water.

The mixture is allowed to remain two or three days at a moderate temperature. A precipitate of tribromophenol ($C_6H_2Br_3OH$) forms, and is collected on a weighed filter, washed with water, and dried in an exsiccator over sulphuric acid to constant weight.

100 parts of tribromophenol correspond to 28·4 parts of phenol. This method is only approximate; the sources of fallacy have been pointed out by Haldane[2] (*see* also p. 742).

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[1] *Zeit. klin. Med.* vol. iii. 1881, p. 465.

[2] *Journal of Physiol.* ix. 218.

CHAPTER XLVI

THE SECRETIONS OF THE SKIN AND ALLIED STRUCTURES

THE secretions of the skin are two in number—the sweat, secreted in the coil of the sweat-glands, and the sebum, secreted by the sebaceous glands that surround the hairs.

THE SWEAT

Physiology of the secretion of sweat.—The sweat-glands are situated in the true skin ; their ducts pass through the epidermis and open on the surface. They are most abundant in man on the palms and soles, and here the greatest amount of perspiration occurs. Different animals vary a good deal in the amount of sweat they secrete, and in the place where the secretion is most abundant. Thus the ox perspires less than the horse and sheep ; perspiration is absent from rats, rabbits, and goats ; pigs perspire mostly on the snout ; dogs and cats on the pads of the feet.

As long as the secretion is small in amount, it is evaporated from the surface at once ; this is called *insensible perspiration*. As soon as the secretion is increased or evaporation prevented, drops appear on the surface of the skin. This is known as *sensible perspiration*. The relation of these two varies with the temperature of the air, the drier and hotter the air, the greater being the proportion of insensible to sensible perspiration. In round numbers the total amount of sweat secreted by a man is two pounds in the twenty-four hours.

The amount of secretion is influenced by two sets of nerves : (1) the vaso-motor nerves ; an increase in the size of the skin-vessels, leading to increased, a constriction of the vessels to diminished perspiration. There are also special secretory fibres (Goltz. Kendall and Luchsinger[2]), stimulation of which causes a secretion even when the circulation is suspended, as in a recently amputated limb. These fibres appear to be contained in the same nerve-trunks as the vaso-motor nerves, as are also the nerve-fibres which supply the plain muscular fibres of the sweat-glands which aid in the expulsion of the secretion. The secretory nerves for the lower limbs are

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[1] *Pflüger's Archiv*, xi. 71.

[2] *Ibid.* xiii. 212.

contained in the sciatic, and are controlled by a centre in the upper lumbar region of the cord; those for the upper limbs lie in the ulnar and median nerves, controlled by a centre in the cervical enlargement of the cord. The secretory fibres for the head pass in the cervical sympathetic, and in some branches of the fifth cranial nerves. These subsidiary centres are dominated by one in the medulla oblongata (Adamkiewicz). These facts have been obtained by experiments on animals (cat, horse). Direct stimulation of the skin causes an increase of sweat, generally bilateral.

The sweat-centres may be excited directly by venous blood, as in asphyxia; or by over-heated blood (over 45° C.), by certain drugs (*see* further); or reflexly by stimulation of the crural and peroneal nerves, or by pungent substances in the mouth, like mustard.

Nervous diseases are often accompanied with disordered sweating; thus unilateral perspiration is seen in some cases of hemiplegia; degeneration of the anterior nerve-cells of the cord may cause stoppage of the secretion. It is sometimes increased in paralysed limbs.

The changes that occur in the secreting cells have been investigated by Renaut in the horse. When charged they are clear and swollen, the nucleus being situated near their attached ends; when discharged they are smaller, granular, and their nucleus is more central.

The sweat, like the urine, must be regarded as an excretion, the secreting cells eliminating substances formed elsewhere.

Composition of the sweat.—Sweat may be obtained in abundant quantities by placing the animal or man in a closed hot-air bath, or from a limb by enclosing it in a vessel made air-tight with an elastic bandage. Thus obtained it is mixed with epidermal scales and a small quantity of fatty matter from the sebaceous glands. The continual shedding of epidermal scales is in reality an excretion. Keratin, of which they are chiefly composed, is rich in sulphur, and, consequently, this is one means by which sulphur is removed from the body.

Observers differ as to the reaction of sweat; some say it is alkaline or neutral, the acidity sometimes observed being due to admixture with fatty acids from the sebaceous glands. Hoppe-Seyler[1] states, however, most positively that the normal reaction is acid, and that the acidity as in the urine is due to acid sodium phosphate. In profuse sweating, however, the secretion usually becomes alkaline or neutral.[2] It has a peculiar and characteristic odour, which varies in different parts of the body, and is due to volatile fatty acids; its taste is saltish, its specific gravity about 1005.

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[1] *Physiol. Chem.* p. 766.

[2] For instance after pilocarpin (Trumpy and Luchsinger, *Pflüger's Archiv*, xviii. 494).

Analyses have been made by numerous observers (Anselmino,[1] Schottin,[2] Favre,[3] L. Wolff,[4] O. Funke,[5] and Leube [6]), and there appear to be great variations in the composition of the sweat. In round numbers the percentage of solids is 1·2, of which 0·9 is organic matter. The following table from Charles' 'Physiological Chemistry'[7] is a compilation from several analyses :—

Water	98·88	per cent.	
Solids	1·12	,,	
Salts	0·57	,,	
NaCl	0·22 to 0·33	,,	
Other salts	0·18	,,	(alkaline sulphates, phosphates, lactates, and potassium chloride)
Fats	0·41	,,	(including fatty acids and cholesterin)
Epithelium	0·17	,,	
Urea	0·08	,,	

The salts are in kind and relative quantity very like those of the urine. Funke was unable to find any urea, but most other observers agree on the presence of a minute quantity. It appears to become quickly transformed into ammonium carbonate. The volatile fatty acids present are formic, acetic, propionic, and butyric.[8] The proteid which, according to Leube, is present, is probably derived from epithelial cells of the epidermis, sweat-glands, and sebaceous glands, which are suspended in the excretion. F. Smith [9] and Leclerc,[10] however, state that in profuse perspiration in the horse there is albumin actually in solution in the sweat.

Abnormal, unusual, or pathological conditions of the sweat. *Drugs.*—Certain drugs (sudorifics) favour sweating, e.g. pilocarpine, Calabar bean, strychnine, picrotoxine, muscarine, nicotine, morphine in small doses, camphor, ammonia. Others diminish the secretion, e.g. atropine, and morphine in large doses.

Large quantities of water, by raising the blood pressure, increase the perspiration.

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[1] Wagner's *Handwörterbuch. d. Physiol.* Art. Haut.

[2] *Arch. f. Physiol. Heilk*, xi. 73.

[3] *Compt. rend.* xxxv. 721.

[4] *Diss.* Greifswald, 1856.

[5] Moleschott's *Untersuch. zur Naturlehre*, iv. 36.

[6] *Arch. f. pathol. Anat.* xlviii. 181; l. 301; *Arch. klin. Med.* vii. 1.

[7] P. 349.

[8] Favre mentions a special acid in addition with formula $C_{10}H_{16}N_2O_{12}$, which he terms sweat-acid, but which requires reinvestigating.

[9] *Veterin. Journ.* Oct. 1888.

[10] *Compt. rend.* cvii. 123.

Some substances introduced into the body reappear in the sweat, e.g. benzoic, tartaric, and succinic acids readily, quinine and iodine with more difficulty (Schottin). Compounds of arsenic and mercury behave similarly (Bergeron and Lemattre[1]).

Diseases.—Cystin has been found in some cases (Gamgee and Dewar[2]); dextrose in diabetic patients (Semmola, Griessinger, Koch, Külz,[3] and many others); bile-pigment in those with jaundice (as evidenced by the staining of the clothes); indigo in a peculiar condition known as chromidrosis (Bizio,[4] Hoffmann[5]); blood or hæmatin derivatives in red sweat; albumin in the sweat of acute rheumatism, which is often very acid; urates and calcium oxalate in gout; lactic acid in puerperal fever, and occasionally in rickets and scrofula.

Kidney diseases.—The relation of the secretion of the skin to that of the kidneys is a very close one. Thus copious secretions of urine, or watery evacuations from the alimentary canal, coincide with dryness of the skin; abundant perspiration and scanty urine generally go together. In the condition known as uræmia, when the kidneys secrete little or no urine, the percentage of urea rises in the sweat; the sputa and the saliva also contain urea under those circumstances. The clear indication for the physician in such cases is to stimulate the skin to action by hot-air baths and pilocarpine, and the alimentary canal by means of purgatives.

Varnishing the skin.—By covering the skin of such an animal as a rabbit with an impermeable varnish, the temperature is reduced, a peculiar train of symptoms set up, and ultimately the animal dies.[6] If, however, cooling be prevented by keeping such an animal in warm cotton-wool, it lives longer. Varnishing the human skin does not seem to be dangerous.[7] Many explanations have been offered to explain the peculiar condition observed in animals; retention of the sweat would hardly do it; the blood is not found *post mortem* to contain any abnormal substance, nor is it poisonous when transfused into another animal. Cutaneous respiration is so slight in mammals (p. 394) that stoppage of this function cannot be supposed to cause death. The animal, in fact, dies of cold; the normal function of the skin in regulating temperature is interfered with by injury to its vaso-motor nerves, and it is only animals with delicate skins which are thus affected. (*See* Animal Heat, Chapter XLVIII.)

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[1] *Central. med. Wiss.* 1864, p. 656.

[2] *Journ. of Anat. and Physiol.* v. 142.

[3] *Text-book on Diabetes*, Marburg, vol. ii. 1875, p. 135.

[4] *Wien. Akad. Sitzungsb.* xxxix. 33.

[5] *Wien. med. Woch.* 1873, p. 292.

[6] Laschkewitsch, *Arch. f. Anat. u. Physiol.* 1868, p. 61.

[7] Senator, *Virchow's Arch.* lxx. 182.

THE SEBUM

When freshly secreted, the sebum is an oily substance, which sets, on cooling, into a white greasy mass. Fatty particles, epithelial cells, and cholesterin crystals are seen on microscopic examination. Its reaction is acid; in addition to fatty matters, it contains proteids, which are two in number; the chief one is, as in milk, casein; there is albumin in addition; sugar is absent. Sometimes when the duct of the sebaceous glands becomes occluded, cysts are formed, and the contents of these have been analysed. Other situations where large quantities of sebum, or substances like it, have been obtained are from the prepuce, and the greasy coating (vernix caseosa) of new-born children. The fatty matter found in the sheep's fleece, that secreted from the coccygeal glands of geese and ducks, and the substance called castoreum (from the beaver's prepuce) come into the same category.

The following tables of analyses[1] give in parts per 1000 the chief results obtained :—

Constituents	Sebaceous cyst (human)[2]	Vernix caseosa (human)[3]	Smegma preputii (human)[3]	Smegma preputii (horse)[3]
Water	317·0	669·8	—	—
Epithelium & proteids	617·5	40	56	—
Fat	41·6	475	528	499
Fatty acids[4] . .	12·1	—	—	—
Alcoholic extract .	—	150	74	96
Aqueous extract . .	—	33	61	54
Ash	11·8	—	—	—

In dermoid cysts which form in ovarian tumours Sotnichewsky[5] found hairs and concretions of calcium carbonate suspended in a fatty mass composed of fats and alkaline soaps. The fats present were chiefly oleic, stearic, and palmitic compounds of an alcohol with high molecular weight. Proteids were present also. The substance is more like sebum than anything else.

The fat of sheep's wool has been chiefly investigated by Schulze.[6] The wool itself comprises from 20 to 40 per cent. of the solids; the amount of fat varies in different kinds of sheep from 7 to 34 per cent. Among the solids are cholesterin and an isomeride of cholesterin, called iso-cholesterin. There is a third alcohol of high molecular weight, which has not been obtained pure. Carius[1] described a compound, which he called hyænic acid ($C_{25}H_{50}O_2$), which may be a derivative of this alcohol.

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[1] Hoppe-Seyler, *Physiol. Chem.* p. 761.

[2] Schmidt, *Deutsch. Arch. klin. Med.* v. 522. Hoppe-Seyler has found leucine and tyrosine in these cysts.

[3] Lehmann, *Gmelin's Handbuch*, viii. 295. Castoreum contains 2 to 8 per cent. of substances soluble in ether. The fat in the smegma is chiefly in the form of ammonium soap. Potash and soda soaps also occur, as well as small quantities of phenol, and in the horse calcium oxalate.

[4] According to Schmidt, these are butyric, valeric, and caproic.

[5] *Zeit. physiol. Chem.* iv. 345.

[6] E. Schulze and Marker, *Journ. prakt. Chem.* cviii. 200; E. Schulze, *Ibid.* N.F. vii. 162; ix. 321; Schulze and Barbieri, *Journ. f. Landwirthsch.* 1879, p. 125. Kossel and Obermüller, *Zeit. physiol. Chem.* xiv. 600.

The secretion of the coccygeal glands of birds has been analysed by de Jonge[2]; he finds it contains 40 per cent. of solids, of which about 15 consist of proteids and nuclein, 19 to 24 of substances soluble in ether, and small quantities of other organic substances and inorganic salts. The proteids are casein and albumin. The substances soluble in ether are chiefly fatty acid (especially oleic acid) compounds, not of glycerin, but of cetyl alcohol; there is also a trace of lecithin. This secretion is used for the lubrication of the feathers. The glands are absent in the running birds, and are most highly developed in water-fowl.

The fatty secretion of the skin of the salamander contains, in addition to fat, proteid, lecithin, and cholesterin, an alkaloid with formula $C_{34}H_{60}N_2O_5$ (Zalesky[3]). The secretion of the skin of most amphibia is a watery one, and appears to be more akin to sweat than sebum. The glands are in structure, however, very different from sweat-glands. The secretion itself has never been thoroughly examined.

Cerumen.—The wax formed in the external auditory meatus appears to be a mixture of sweat and a secretion from certain glands similar in structure to sebaceous glands. Petrequin and Chevalier[4] find it contains 10 to 11 per cent. of water, 26 to 30 per cent. of fat, 40 to 50 per cent. of potassium soaps, and traces of inorganic salts. Its reddish pigment has not been examined.

The secretion of the Meibomian glands of [illegible] eyelid, which are similar in structure to the sebaceous glands, and also that of the sebaceous glands round the eyelashes, become mixed. That of the Meibomian glands appears to be the less watery secretion of the two.

Tears, the secretion of the lacrymal glands, have been but little investigated. The secretion is more akin to saliva than to that of any other glands; and the lacrymal gland is in structure like a salivary gland. It is, however, convenient to mention them in this place. Stimulation of the fifth cranial nerve produces a clear, that of the sympathetic a cloudy, more alkaline secretion (compare Salivary Glands, p. 617). Lerch found in 1000 parts by weight of tears 980 parts of water, 13 of sodium chloride, and 5 of proteids. The chief proteid appears to be a globulin, as when dropped into water the tears give a cloudy precipitate.

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[1] *Ann. Chem. Pharm.* cxxix. 168.

[2] *Zeit. physiol. Chem.* iii. 225.

[3] Hoppe-Seyler's *Med. chem. Unters.* Heft i. p. 109.

[4] *Compt. rend.* lxviii. No. 16; lxix. No. 19.

PART VI

GENERAL METABOLISM

CHAPTER XLVII

EXCHANGE OF MATERIAL

THE word *metabolism* has been often employed in the preceding chapters, and, as there explained, it is used to express the sum total of the chemical exchanges that occur in living tissues. The chemical changes have been considered separately under the headings Alimentation, Excretion, Respiration, &c. We have now to put our knowledge together, and consider these subjects in their relation to one another.

The living body is always giving off by the lungs, kidneys, and skin the products of its combustion, and is thus always tending to lose weight. This loss is compensated for by the intake of food, and of oxygen. For the material it loses, it receives in exchange fresh substances. If, as in a normal adult, the income is exactly equal to the expenditure, the body-weight remains constant. If, as in a growing child, the income exceeds the expenditure, the body gains weight; and if, as in febrile conditions, or during starvation, the expenditure exceeds the income, the body wastes.

The first act in the many steps which constitute metabolism is the taking of food, the next digestion of that food, the third absorption, and the fourth assimilation. Food, diet, digestion, and absorption have already been dealt with, and it is only necessary to refer the reader to the chapters where these are described. In connection with these subjects, it is important to note the necessity for a mixed diet, and the relative and absolute quantities of the various proximate principles which are most advantageous. Assimilation is a subject which is exceedingly difficult to describe; it is the act of the living tissues in selecting, appropriating, and making part of themselves the substances brought to them by the nutrient blood-stream from the lungs on the one hand, and the alimentary canal on the other. The chemical processes involved in some of these transactions have been already dwelt on in connection with the functions of the liver and other secreting organs, but even there our information on the subject is limited; much more is this the case in connection with other tissues. The interesting theory of Pflüger, in connection with the behaviour of the nitrogen in a food-proteid when it becomes part of a living proteid, should be read also in this connection (p. 115).

The functions which we call digestion, absorption, and assimilation are the three steps in nutrition, or the building up of the living tissues; these may, to use Gaskell's expression, be spoken of as *anabolic.*

Supposing the body to remain in the condition produced by these anabolic processes, what is its composition? A glance through the chapters on the cell, the blood, the tissues, and the organs will convince the inquirer that different parts of the body have very different compositions; still, speaking of the body as a whole, Volckmann and Bischoff state that it contains 64 per cent. of water, 16 of proteids (including gelatin), 14 of fat, 5 of salts, and 1 of carbohydrates. The carbohydrates are thus the smallest constituent of the body; they are the glycogen of the liver and muscles, and small quantities of inosite, and dextrose in various parts.

The most important, because the most abundant, of the tissues of the body is the muscular tissue. Muscle forms about 42 per cent. of the body-weight,[1] and contains, in round numbers, 75 per cent. of water and 21 per cent. of proteids; thus about half the proteid material and of the water of the body exist in its muscles.

The body, however, does not remain in this stable condition; even while nutrition is occurring, destructive changes are taking place simultaneously; each cell may be considered to be in a state of unstable equilibrium, undergoing *anabolic*, or constructive processes, on the one hand, and destructive, or *katabolic*, processes on the other. The katabolic series of phenomena commences with combustion; the union of oxygen with carbon to form carbonic acid, with hydrogen to form water, with nitrogen, carbon, and hydrogen to form urea, uric acid, creatinine, and other less important substances of the same nature. The formation of these last-mentioned substances, the nitrogenous metabolites, is, however, as previously pointed out, partly synthetical. The discharge of these products of destructive metabolism by the expired air, the urine, the sweat, and fæces is what constitutes excretion; excretion is the final act in the metabolic round, and the composition of the various excretions have been considered in some of the later chapters of this book.

An examination of the intake (food and oxygen) and of the output (excretions) of the body can be readily made; much more readily, it need hardly be said, than an examination of the intermediate steps in the process. A contrast between the two can be made by means of a balance-sheet. A familiar comparison may be drawn between the affairs of the animal body and those of a commercial company. At the end of the year the company presents a report in which its income and its expenditure are contrasted on two sides of a balance-sheet. This sheet is a summary of the monetary affairs of the undertaking; it gives few details, it gives none of the intermediate steps of the manner in which the property has been employed. This is given in the preliminary parts of the report, or may be entered into by still further examining the books of the company.

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[1] The following is in round numbers the percentage proportion of the different structural elements of the body: skeleton, 16; muscles, 42; fat, 18; viscera, 9; skin, 8; brain, 2; blood, 5.

In the parts of this book that precede this chapter I have endeavoured to give an account of the various transactions that occur in the body. I now propose to wind up by presenting a balance-sheet. Those who wish still further to investigate the affairs of the body may do so by the careful study of works on physiology; still, text-books and monographs, however good, will teach one only a small amount; the rest is to be learnt by practical study and research; and we may compare physiologists to the accountants of a commercial enterprise, who examine into the details of its working. Sometimes, in business undertakings, a deficit or some other error is discovered, and it may be that the source of the mistake is only found after careful search. Under these conditions, the accountants should be compared to physicians, who discover that something is wrong in the working of the animal body; and their object should be to discover where, in the metabolic cycle, the mistake has occurred, and subsequently endeavour to rectify it.

The construction of balance-sheets for the human and animal body may be summed up in the German word *Stoffwechsel*, or, as Dr. Burdon-Sanderson[1] translates it, '*exchange of material.*' A large number of investigators have applied themselves to this task, and from the large mass of material published, I shall only be able to select a few typical examples. The subject has been worked out specially by the Munich school, under the lead of Pettenkofer and Voit.

The necessary data for the construction of such tables are:—

(1) The weight of the animal before, during, and after the experiment.

(2) The quantity and composition of its food.

(3) The amount of oxygen absorbed during respiration.

(4) The quantity and composition of urine, fæces, sweat, and expired air.

(5) The amount of work done, and the amount of heat developed. (The subject of animal heat will be considered in a separate chapter.)

Water is determined by subtracting the amount of water ingested as food from the quantity lost by bowels, urine, lungs, and skin. The difference is a measure of the combustion of hydrogen.

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[1] *Syllabus of Lectures*, 1879.

Nitrogen.—The nitrogen is derived from proteids and albuminoids, and appears chiefly in the urine as urea and uric acid. Minute quantities are eliminated as similar compounds in sweat and fæces. From the amount of nitrogen so found, the amount of proteids which have undergone combustion is calculated. Proteids contain, roughly, 16 per cent. of nitrogen; so 1 part of nitrogen is equivalent to 6·3 parts of proteid; or 1 gramme of nitrogen to 30 grammes of flesh (Voit).

Fat.—Subtract the carbon in the metabolised proteid (proteid contains 54 per cent. of carbon) from the total carbon eliminated by lungs, skin, bowels, and kidney, and the difference represents fat that has undergone metabolism. Fat contains 76·5 per cent. of carbon; hence the carbon, which represents fat, multiplied by 1·3, gives the amount of fat which has undergone combustion.

The Discharge of Carbon

The influence of food on the rate of discharge of carbonic acid is immediate. The increase after each meal, which may amount to 20 per cent., reaches its maximum in about one or two hours. This effect is most marked when the diet consists largely of carbohydrates.

About 95 per cent. of the carbon discharged leaves the organism as carbonic acid. The total insensible loss (=carbonic acid + water given off − oxygen absorbed) amounts in man to about 25 grammes per hour. Of this total hourly discharge of carbonic acid, less than 0·5 per cent. is cutaneous. The hourly discharge of carbonic acid in a man at rest is about 32 grammes, the weight of oxygen absorbed being 25 to 28 grammes in the same time. The hourly discharge of watery vapour is about 20 grammes.

As a volume of carbonic acid (CO_2) contains the same weight of oxygen as an equal volume of oxygen (O_2), it is obvious that, if all the absorbed oxygen were discharged as carbonic acid, the 'respiratory quotient' (by volume) $= \frac{CO_2 \text{ expired}}{O_2 \text{ absorbed}}$ would be equal to 1. This, however, is not the case, the volume of oxygen absorbed being in excess of the carbonic acid discharged. In animals which feed exclusively on carbohydrates (this would only be possible for a short time) equality is approached. The excess of oxygen is greatest when the diet consists largely of fats.

On a mixed diet, comprising 100 grammes of proteid, 100 of fat, and 250 of carbohydrates, with a carbonic acid discharge of 770 grammes daily, and a daily assumption of 666 grammes of oxygen, 560 grammes of the oxygen are discharged in the carbonic acid, about 9 in urea, and 97 grammes in the form of water (of which 78 grammes are formed from the hydrogen of the fat); the respiratory quotient is then 0·84. In hibernation the respiratory quotient sinks lower than in any other known condition (often less than 0·5), for the animal then lives almost entirely on its own fat. The discharge of carbonic acid is increased by muscular work, and the respiratory quotient also rises. Diminution of the surrounding temperature causes increased discharge of carbonic acid. (These points are all discussed more fully in Chapter XIX.)

The Discharge of Nitrogen

In man the minimum daily allowance of nitrogen is 15 grammes, or 0·02 per cent. of the body-weight: in the carnivora about 0·1 per cent.; in the ox, as an instance of a herbivorous animal, 0·005 per cent. In certain races of mankind (e.g. coolies) the nitrogen requirement is less than in Europeans. The reason why this is so is not understood. The bearing of this fact on vegetarianism is pointed out in the chapter on food (p. 599).

Some recent experiments by Hirschfeld[1] have shown that for a short time nitrogenous equilibrium can be maintained on a smaller daily supply of nitrogen than 15 grammes. Experiments extended over a longer time have previous to this shown that sooner or later the body begins to waste if the 15 grammes daily are not supplied in the food.

In an animal fed exclusively on flesh the discharge of nitrogen at first increases *pari passu* with the absorption of proteid, the absorption of oxygen being proportionately increased at the same time. The animal, however, gains weight from increase of fat, the proteid being split into what is called a nitrogenous moiety, which is burnt off, and a non-nitrogenous moiety which is converted into fat.

The discharge of nitrogen is but little influenced by muscular work (*see* p. 436); the increased combustion that occurs in working as compared with resting muscles falls on their non-nitrogenous constituents. The questions of the nutritive value of gelatin, the origin of fat from proteids and carbohydrates, and the conditions of nitrogenous discharge in starvation, fever, and other disordered conditions will be dealt with later in special sections.

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[1] *Pflüger's Archiv*, xli. 533.

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Balance of Income and Discharge in Health

In Chapter XXVII (p. 604) tables are given of adequate diets; these will in our balance-sheets represent the source of income; the other side of the balance-sheet the expenditure consists of the excretions.

Exchange of material on an adequate diet (Ranke's table).[1]

Income			Expenditure		
Foods	Nitrogen	Carbon	Excretions	Nitrogen	Carbon
Proteid, 100 gr.	15·5 gr.	53·0 gr.	Urea, 31·5 gr. Uric acid, 0·5 „	14·4	6·16
Fat, 100 „	0·0 „	79·0 „	Fæces . . .	1·1	10·84
C'rb'hyd'at's, 250 „	0·0 „	93·0 „	Respiration (CO_2) .	0·0	208·00
	15·5 „	225·0 „		15·5	225·00

In man the discharge of nitrogen per kilo. of body-weight is 0·21 gramme, and of carbon 3·03 grammes, the quotient $\frac{C}{N} = 14{\cdot}5$. In carnivorous animals, which, according to Bidder and Schmidt, use 1·4 N and 6·2 C per kilo. per diem, $\frac{C}{N} = 4{\cdot}4$. In the human being on a flesh diet $\frac{C}{N} = 5{\cdot}2$, the exchange thus approaching the condition of the carnivora. This is illustrated by the following balance-sheet (Ranke):—

Income			Expenditure		
	Nitrogen	Carbon		Nitrogen	Carbon
Food . .	62·3 gr.	279·6	Discharged by excretion . .	44·0	263·0
Disintegration of tissues . .	—	45·9	Retained in store .	18·3	62·5
	62·3	325·5		62·3	325·0

The details of the above experiment may be given as illustrating the method of working out a problem in exchange of material: 1832 grammes of meat used as food yielded 3·4 per cent. of nitrogen, i.e. 62·3 gr., and 12·5 per cent. of carbon, i.e. 229·3 gr.; 70 gr. of fat added to the food yielded 72 per cent. of carbon, i.e.

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[1] The above table was constructed from data derived from the observations of Prof. Ranke on himself. For it I am indebted to Prof. Sanderson's *Syllabus of Lectures*, which is also the source of most of the statements in the *résumé* of the chief facts relating to the discharge of carbon and nitrogen just given.

50·3 gr.: 229·3 + 50·3 = 279·6 = total carbon in food. During the same period 86·3 gr. of urea were discharged, containing 46·6 per cent., i.e. 40·4 gr. of nitrogen, and 20 per cent., i.e. 17·3 gr. of carbon, to which must be added 2 gr. of uric acid, containing 33 per cent., i.e. 0·66 gr. of nitrogen, and 35 per cent., i.e. 0·7 gr. of carbon. Further, 2·9 gr. of nitrogen and 14 gr. of carbon were discharged in the fæces, and 231 gr. of carbon as carbonic acid in the expired air. Hence the total discharge of nitrogen = 40·4 + 0·66 + 2·9 = 44 gr., and the total discharge of carbon = 17·3 + 0·7 + 14 + 231 = 263 gr. Deducting the quantity of nitrogen discharged from that taken in, 18·3 gr. must have been retained in the body, as 108 gr. of proteid, and consequently 53 per cent. of that weight = 62·5 gr. of carbon, were also retained. Comparing the quantity of carbon disposed of in the twenty-four hours with the quantity introduced as food, we find the latter is in excess by 45·9 gr., which must have been derived from the disintegration of the fat of the body.

Another table of exchange of material on adequate diet may be quoted from the work of Pettenkofer and Voit. This takes into account the elimination of water as well as of carbon and nitrogen. In the first experiment the man did no work.

Income			Expenditure			
Food	Nitrogen	Carbon	Excretions	Nitrogen	Carbon	Water
Proteid, 137 gr	19·5	315·5	Urine	17·4	12·7	1279
Fat, 117 „			Fæces	2·1	14·5	83
Carbohydrate, 352 „			Lungs	—	248·6	828
Water, 2016 „	—	—				
				19·5	275·8	2190

Here the body was in nitrogenous equilibrium, and it eliminated more water than it took in by 174 grammes, this being derived from oxidation of hydrogen. It stored 39·7 grammes of carbon, which is equivalent to 52 grammes of fat.

The next table gives the results of an experiment on the same man on the same diet, but who did active muscular work during the day :—

Expenditure	Nitrogen	Carbon	Water
Urine . . .	17·4	12·6	1194
Fæces . . .	2·1	14·5	94
Lungs . . .		309·2	1412
	19·5	336·3	2700

It is important to notice that the discharge of nitrogen was unaltered, while that of both carbon and hydrogen was increased.

Inanition

The conditions of metabolism, both as regards exchange of material and the production of heat during starvation, have been investigated in animals by Collard de Martigny,[1] Chossat,[2] C. Schmidt,[3] Schuchardt,[4] Frerichs,[5] Bischoff and Voit[6]; in human beings by Pettenkofer and Voit,[7] J. Ranke,[8] Schultzen,[9] Seegen,[10] Falck,[11] and Schimanski.[12]

The income from without is, under these circumstances, *nil*: expenditure still goes on, as a result of the disintegration of the tissues; the amount of disintegration is measured by the discharges in the manner already described. The following table from Ranke's experiment on himself represents the exchange for a period of twenty-four hours, twenty-four hours having elapsed since the last meal.

Income			Expenditure		
Disintegration of tissue	Nitrogen	Carbon	Excretions	Nitrogen	Carbon
Proteid, 50 gr.	7·8	26·5	Urea, 17 gr. Uric acid, 0·2 gr.	7·8	3·4
Fat, 199·6 gr.	0·0	157·5	Respiration (CO_2)	0·0	180·6
	7·8	184·0		7·8	184·0

The discharge of nitrogen per kilo. of body-weight was reduced to 0·1, $\frac{C}{N}$ being 23·5. In carnivorous animals: in prolonged inanition, the discharge of nitrogen per kilo. is 0·9, and $\frac{C}{N} = 6{\cdot}6$.

During starvation the man or animal gradually loses weight, the temperature, after a preliminary rise, sinks; the functions get weaker by degrees, and ultimately death ensues, the total weight lost being from 0·3 to 0·5 of the original body-weight.

The age of the animal influences the time at which death occurs, old animals withstanding the effects of hunger better than young ones.

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[1] *Journ. de physiol. expérim. et path.* vol. viii. 1828, p. 152.

[2] 'Recherches sur l'inanition,' *Mém. de l'Acad. Roy. des Sciences*, vol. viii. Paris, 18

[3] Bidder and Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, Mitau and Leipzig, 1852, p. 292.

[4] *Diss.* Marburg, 1847.

[5] *Arch. für Anat. u. Physiol.* 1848, p. 469

[6] Bischoff and Voit, *Die Gesetze der Ernärung des Fleischfressers*, Leipzig and Heidelberg, 1860, p. 42; *Zeit. Biol.* ii. 307; v. 369.

[7] *Ibid.*

[8] *Arch. f. Anat. u. Physiol.* 1862, p. 311.

[9] *Ibid.* 1863, p. 31.

[10] *Wien. Akad. Sitzungsb.* March 16, 1871.

[11] *Beiträge zur Physiol. Hygiene, &c.* vol. i. Stuttgart, 1875.

[12] *Zeit. physiol. Chem.* iii. 396.

This statement was originally made by Hippocrates, and was borne out by the experiments of Martigny and Chossat. Young animals lose weight more quickly, and die after a smaller loss of weight, than old ones.

The excretion of nitrogen falls quickly at the commencement of an experiment; it reaches a minimum which remains constant for several days; it then rises when the fat of the animal has been used up, and then quickly falls with the onset of symptoms of approaching death (Voit, Falck, Schmidt, Schimanski).

The sulphates and phosphates in the urine show approximately the same series of changes (Bidder and Schmidt).

The discharge of carbonic acid and the intake of oxygen fall, but not so quickly as the body loses weight; it is not until quite the last stages that these are small in proportion to one another.

The fæces become smaller and smaller in quantity until no discharge from the rectum occurs at all.

The amount of bile secreted also falls; but bile is found in the gall-bladder and intestine after death.

Chossat, Schuchardt, Schmidt, and Voit have constructed tables which show the loss of weight that occurs in different organs. Taking the total loss of weight as 100, the loss due to that of individual organs may be stated as follows (Voit):—

Bone	5·4	Pancreas	0·1	Brain and cord	0·1
Muscle	42·2	Lungs	0·3	Skin and hair	8·8
Liver	4·8	Heart	0·0	Fat	26·2
Kidneys	0·6	Testes	0·1	Blood	3·7
Spleen	0·6	Intestine	2·0	Other parts	5·0

Some organs thus lose but little weight; the loss of weight is greatest in the muscles, fat, skin, liver, and blood. Of the muscles, the great pectorals waste most (Chossat). Demant[1] found an increase of creatine and a diminution of lactic acid in the muscles of starving birds.

The following are Chossat's observations on the body-temperature in pigeons:—

Condition of animals	Temperature	
	Midday	Midnight
Healthy pigeons	42·22° C.	41·48° C.
1st third of starvation period	42·11°	39·8°
2nd ,, ,, ,,	41·87	38·7°
3rd ,, ,, ,,	41·37°	37·3°

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[1] *Zeit. physiol. Chem.* iii. 381.

There is thus the greatest fall of temperature in the night—that is, when even under normal circumstances the temperature and the vitality of the body are least. The frequency of respiration and the discharge of carbonic acid run parallel to the temperature. Falck obtained a similar result in dogs. Death may be delayed somewhat by artificial warmth, but ultimately occurs from asthenia, sometimes accompanied by convulsions.

Exchange of Material with various Diets

The reasons why a mixed diet is necessary have been already explained (p. 602). Numerous experiments have, however, been made in the study of metabolism on abnormal diets.

Feeding with meat.—The chief facts concerning this form of nutrition in regard to man have been stated on p. 603. The same is in the main true for animals. The principle that underlies Banting's method of treating obesity is to give meat almost exclusively: the individual then derives the additional supply of carbon necessary for combustion from his own adipose tissue. We have already seen that this may be and often is counteracted by the laying on of fat which comes from the non-nitrogenous moiety of the proteid.

Feeding with fat.—If an animal receives fat only, the nitrogenous excreta are derived from the disintegration of tissue without any corresponding quantity of nitrogen being supplied in exchange in the food. When fat only is given, or a large excess of fat exists in the food, the respiratory quotient falls. By feeding dogs with a mixture of fat and flesh, Pettenkofer and Voit's[1] experiments gave such inconsistent results that no conclusion can be drawn from them F. Hofmann,[2] however, was more successful. After a period of inanition a dog was fed on a mixture of a large amount of fat and a small amount of proteid. After death the quantity of fat found in the body was such that only a small part could have been derived from the proteid, the greater amount being directly derived from the fat of the food. The animal, moreover, lays on fat in which palmitin, stearin, and olein are mixed in a definite proportion; this proportion is often different in the fat of the food. In addition to this an animal will fatten (laying on fat with its usual composition) on fatty food, such as spermaceti, which contains no glycerides.

Feeding with carbohydrates.—The respiratory quotient approaches unity when carbohydrates alone are taken. So far as regards nitrogen the animal is in a state of inanition, as when fat alone is taken. If given in combination with other foods, both carbohydrates and fat act as proteid-sparing foods (see p. 603).

The table on the next page is from Pettenkofer and Voit,[3] and illustrates what happens in a dog on a mixed diet of flesh and carbohydrates.

Even when the diet consists wholly of carbohydrates, fat is laid on; the fat laid on when meat and starch are both present in the food comes partly from the

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[1] *Zeit. Biol.* ix. 30.

[2] *Ibid.* viii. 153.

[3] *Ibid.* ix. 435.

Food				Changes in the body			Fat		
Flesh	Starch	Sugar	Fat	Amount of proteid decomposed calculated from urea excreted	Proteid gained or lost by the body	Amount of carbohydrates decomposed	From fat of food	Lost from the body	Derived from food other than fat
0	379	—	17	211	−211	379	+17		24
0	608	—	22	193	−193	608	+22		22
400	210	—	10	436	− 36	210	+10	− 8	—
400	-	227	—	393	+ 7	227	—	−25	—
400	344		6	413	− 13	344	+ 6		39
500	167	—	6	530	− 30	167	+ 6		8
500	—	182	—	537	− 37	182	—		16
800	379	—	14	608	+192	379	+14		55
1500	172	—	4	1475	+ 25	172	+ 4		43
1800	379	—	10	1469	+331	379	+10		112
2500		-		2512	+ 12	0	—		57

proteid and partly from the carbohydrate of the food. When no carbohydrate is given at all, as in the last experiment, the nitrogenous metabolism is raised. Carbohydrate food is thus when given with other foods both fat-sparing and proteid-sparing. It is difficult on chemical grounds to explain the formation of fat from carbohydrates. The fact was first observed in pigs by Lawes and Gilbert, and has since been confirmed by numerous investigators.[1]

The origin of fat.—Prof. Foster[2] thus sums up the conclusions he has arrived at from a study of the chief researches on this subject :—

1. Fat is actually formed in the body, and is not exclusively, if at all, fat merely stored up from the fat of the food.[3]

2. The carbon elements of the newly formed fat may be supplied either from carbohydrate food, or from the carbon surplus of proteid food, or from fats taken as food which are not the natural constituents of the body fat.

One of the most important instances of the carbohydrate origin of fat is the formation of bees'-wax. A chemical link between carbohydrates and fats is the fact that butyric acid is obtainable from starches and sugars (p. 103).

Instances of the formation of fat from proteids are (1) the laying on of fat in carnivorous animals ; (2) the formation of adipocere (p. 426) ; (3) the gradually increasing quantity of fat in old cheeses.

The most striking examples of the formation of fat by intracellular metabolic processes is seen in fatty degeneration, and in that special form of this degeneration that occurs in the formation of milk. The blood contains a mere trace of fat, so milk formation is no mere filtration process. The food may, as in the case of cows, contain little or no fat.

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[1] Among the more recent may be mentioned Meissl, *Zeit. Biol.* xxii. 63 ; and Rubner, *Ibid.* p. 272.

[2] *Text-book*, 5th edit. p. 776. The chief references to literature relating to this subject will be found in a paper by Voit, *Zeit. Biol.* v. 79.

[3] Compare Bunge's view, p. 706. *See* also Hofmann's experiments, p. 886.

3. The fat stored up appears as granules or drops deposited in the cell-substance, and the increase of fat in the cells is accompanied first by a growth, and subsequently by a consumption of the cell-substances.

Feeding with gelatin.—A diet containing gelatin alone will not support life This fact is somewhat remarkable when one considers the closely allied chemical nature of gelatin and proteids. When gelatin alone is given the body wastes, and the urea excreted is diminished, as in inanition. If an enormous amount of gelatin is given the urea increases. Gelatin, however, like carbohydrates and fats, appears to be a 'proteid-sparing' food, and if given mixed with proteids seems to protect the proteids from oxidation. Gelatin can thus be substituted for a part of the proteid in the food.

The following table is much abbreviated from the fuller ones given by Voit[1]; it illustrates the facts mentioned above:—

Food			Daily loss or gain in grammes of flesh
Meat	Fat	Gelatin	
0	0	0	−338
0	200	200	−105
200	200	200	−124
300	200	200	+ 32
300	200	100	− 84
500	200	0	−136

The dog experimented on weighed 40 to 50 kilos. Without food it lost 338 grammes of flesh daily; 500 grammes of meat with fat was an insufficient diet for the animal, for even then it lost 136 grammes of flesh. But 300 grammes of meat with 200 of gelatin were sufficient for it; it even gained weight. Gelatin is largely used in the form of jellies in the sick-room. Its value appears to be, not that it is itself nutritious, but that mixed with proteid food it enables the patient to get on with less proteid, owing to the sparing action it exercises on proteid metabolism.

Feeding with peptones.—In the present day, when artificially digested foods are so much employed, it is of great importance that their nutritive value should be known. Here experimental and clinical evidence coincide in a most favourable way in relation to their nutritive value. The first experiments on this subject were made by Plósz[2] and Maly.[3] As our knowledge since that time has advanced on the subject of albumoses and peptone, I shall quote only a recent experiment: the results are, however, similar to those of earlier investigators.

Pollitzer[4] fed a dog for successive periods on meat, peptone, proto-albumose, hetero-albumose, and gelatin. The table on the following page gives the results of nitrogen estimations in food and excreta.

It will be seen that peptones and albumoses have the same nutritive value as meat, this result contrasting with the loss of nitrogen and body-weight when gelatin is employed.

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[1] *Zeit. Biol.* viii. 330; and Voit and Hofmann, *ibid.* p. 347.

[2] *Pflüger's Archiv*, ix. 323. [3] *Ibid.* p. 585. [4] *Ibid.* xxxvii. 301.

Diet	Number of days	Nitrogen		
		In food	In urine & fæces	Gain or loss
1. Meat . . .	6	2·4 gr.	1·9 gr.	+ 0·5 gr.
2. Peptone . . .	2	2·4 „	1·8 „	+ 0·6 „
3. Meat . . .	3	2·4 „	1·9 „	+ 0·5 „
4. Proto-albumose .	2	2·4 „	1 8. „	+ 0·6 „
5. Hetero-albumose .	1	2·5 „	1·7 „	+ 0·8 „
6. Meat . . .	4	2·1 „	1·7 „	+ 0·4 „
7. Gelatin . . .	3	2·3 „	2·8 „	− 0·5 „
8. Meat . . .	4	2·1 „	1·7 „	+ 0·4 „

Effect of Varying External Conditions on Exchange of Material

Effect of atmospheric temperature.—In warm-blooded animals the effect of a low surrounding temperature is to increase katabolism, or combustion in the body; the body loses more heat, and therefore more must be produced to keep the animal's temperature within normal limits. The effect of a rise of atmospheric temperature is the reverse. The effect of cooling the skin and the corresponding increase in metabolism are well shown by an observation of Weiske's[1] on the shearing of sheep. After shearing they excreted daily 1 gramme of urea more than before shearing. In cold-blooded animals, i.e. animals whose temperature varies with that of the surrounding atmosphere, a rise or fall of the latter is accompanied respectively with a rise or fall of combustion in the body.

Influence of light (*see* p. 211).

Alterations of body-temperature.—If the changes of the external temperature are so great as to cause a rise (as in steam-baths—Bartels,[2] Naunyn,[3] Scheich[4]) or a fall (as in hibernation) of body-temperature, the metabolic changes are increased and decreased respectively as in cold-blooded animals.

Effect of compressed and rarefied air.—The influence of these factors on the respiratory exchanges has been described on p. 378. Experiments relating to their effect on the discharge of urea are contradictory, Bert[5] and Hadra[6] finding that compressed air caused an increase in the excretion of urea, Fränkel[7] finding no such increase. In rarefied air Fränkel found in some of his experiments an increase, in others no increase in the urea excreted. In dyspnœic conditions, where the supply of oxygen is deficient, the urea increases, and the respiratory quotient $\frac{CO_2}{O_2}$ becomes greater than unity (Fränkel,[8] Herter[9]).

Effect of removal of blood from the body. The chief effect of a removal of blood from the body is the speedy formation of new blood-corpuscles. The intake of oxygen and discharge of carbonic acid are lessened, and the output of urea is increased (Bauer,[10] Jolyet and Regnard[11]). The menstrual flow and epistaxis in strong, healthy people cause no alteration in exchange of material.

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[1] Hoppe-Seyler's *Med. chem. Unters.* Heft iii. p. 418.

[2] *Pathol. Untersuch.* 1864.

[3] *Arch. f. Anat. u. Physiol.* 1870.

[4] *Arch. f. exper. Path.* iv. 82.

[5] *La pression barométrique*, Paris, 1878, p. 823.

[6] *Diss.* Strasburg, Berlin, 1879.

[7] *Zeit. klin. Med.* ii. 1.

[8] *Centr. med. Wiss.* 1875, No. 44.

[9] Hoppe-Seyler's *Physiol. Chem.* p. 954.

[10] *Zeit. Biol.* viii. 567.

[11] *Gaz. méd. de Paris*, 1877, pp. 179, 190.

Effect of increasing the volume of blood.—The injection of the blood from one animal into that of another causes a greater or less destruction of the first animal's blood-corpuscles. The effect, however, varies much with the species of animal used,[1] and appears to be due to the solvent effect of the second animal's serum; it is especially marked when the two animals belong to different species. The effect on the urine is a slight increase in the discharge of urea; the effect is rather greater when serum instead of defibrinated blood is injected (Forster[2]).

Exchange of Material under the Influence of Organic and Inorganic Substances used as Foods, Drugs, or Poisons

Lactic, acetic, tartaric, and succinic acids, asparagine, and glycerin are oxidised in the body to form carbonic acid and water, and, like carbohydrates and fat, are 'proteid-sparing' materials (Hoppe-Seyler,[3] Weiske,[4] Lewin[5]). Glycerin passes partly as such into the urine (Tschirwinsky[6]). Glycerin increases the liver glycogen (Weiss, Luchsinger, Salomon; *see* p. 543). Pure glycerin increases the excretion of uric acid (Horbaczewski[7]).

Phenylacetic acid produces increased proteid metabolism (Salkowski[8]).

Alcohol in small quantities diminishes (Beck and Bauer[9]), in large doses increases the output of carbonic acid (Parkes[10]). The effect on urea is small or none at all (Parkes, Munk[11]).

Coffee produces the same effects as alcohol (Hoppe-Seyler,[12] Voit[13]). The effect of various drugs on the output of urea is given on p. 724.

Water stimulates metabolic activity, and also assists in washing out the products of metabolism from the tissues to the place where they are excreted.

Sodium chloride is also essential for the due discharge of metabolic function (*see* also p. 61).

Phosphates appear to be equally necessary (*see* pp. 62, 256).

Calcium and potassium salts, especially the former, are normal constituents of the body, and without a due supply of these the body wastes.

Phosphorus-poisoning is accompanied with a rapid fatty degeneration of the liver, and the appearance of leucine and tyrosine in the urine. The body wastes quickly. There is an increased output of nitrogen, both as urea and uric acid (Storch,[14] Bauer,[15] Fränkel[16]).

Arsenic and antimony poisoning produce the same effects, but in a much less marked manner (v. Boeck,[17] Weiske,[18] Gähtgens,[19] Kossel[20]). In very small doses antimonious oxide produces little or no increase in the discharge of urea

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[1] *See* E. A. Schäfer, *Report on Transfusion*, *Trans. Obstet. Soc.* xxi.

[2] *Sitzungsb. d. Bayer. Akad. d. Wiss.* July 3, 1875.

[3] *Physiol. Chem.* p. 957.

[4] *Zeit. Biol.* xv. 261 (on asparagine).

[5] *Ibid.* p. 243. *See* also Munk, *Arch. pathol. Anat.* lxxvi. 119 (on glycerin).

[6] *Zeit. Biol.* xv. 252.

[7] *Monatsh. Chem.* vii. 105.

[8] *Zeit. physiol. Chem.* xii. 267.

[9] *Zeit. Biol.* x. 361.

[10] *Proc. Roy. Soc.* 1870, Nos. 120 and 123.

[11] *Verhandl. d. physiol. Ges. Berlin*, 1879, No. 6.

[12] *Physiol. Chem.* p. 958.

[13] *Unters. u. d. Einfluss d. Kochsalz, Kaffee, &c.* München, 1860, p. 67.

[14] *Der acuten Phosphorgiftung*, Copenhagen, 1865.

[15] *Zeit. Biol.* vii. 63; xiv. 527.

[16] *Zeit. physiol. Chem.* iv. 430.

[17] *Zeit. Biol.* xii. 512.

[18] *Journ. f. Landwirthsch.* xxiii. 317.

[19] *Centr. med. Wiss.* 1876, pp. 321, 833.

[20] *Arch. f. exper. Path.* v. 128.

(Chittenden and Blake[1]). In small doses arsenic diminishes the excretion of carbonic acid (Chittenden and Cummins[2]). Among other substances that similarly diminish the output of carbonic acid, Chittenden and Cummins place uranium salts, copper sulphate, and tartar emetic; morphine, quinine, and cinchonidine having little or no effect. Ferric chloride, according to Rabuteau,[3] increases the excretion of urea, according to Munk[4] it does not.

Exchange of Material in Diseases

Fever.—Fever is a condition in which the temperature of the body is raised above the normal, and the degree to which it is raised is a measure of the intensity of the febrile condition. A rise of temperature may be produced either by increased production of heat, due to the increase of katabolic processes in the body, or to a diminished loss of heat from the body. A mere increase in the production of heat does not necessarily produce fever. By administering an excess of food, combustion is increased in the body: but in the healthy individual this does not produce a rise of temperature, because *pari passu* with the increased production, there is increased loss of heat. Similarly, diminution in the loss of heat, such as occurs on a hot as compared with a cold day, does not produce fever, because the production of heat within the body is correspondingly diminished. In fever there is increased production of heat, as is seen by the study of exchange of material; the intake of food is, as a rule, very small; the discharge of nitrogen and carbon results from the disintegration of tissues, which, as compared with that in simple inanition, is large; the tissues are said to be in a labile condition, that is, they are easily broken down. In most febrile states, the skin is dry, the sweat-glands, like most of the secreting organs of the body, being comparatively inactive, and so the discharge of heat is lessened. The skin may, however, sometimes be bathed in perspiration, and yet high fever be present. The essential cause of the high temperature is neither increased formation nor diminished discharge of heat, but an interference with the reflex mechanism, which is health, operates so as to equalise the two.

Increased nitrogenous metabolism in fever has been observed by Huppert and Riesell[5] in pneumonia, by Schimanski[6] in pyæmic conditions, by Naunyn[7] and Sydney Ringer[8] in other febrile conditions. Ringer showed the correspondence in temperature and output of nitrogen very clearly in intermittent fever (ague).

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[1] *Studies from Lab. Physiol. Chem. Yale Univ.* ii. 87.

[2] *Ibid.* 200.

[3] *Compt. rend.* lxxxvi. 1169.

[4] *Verhandl. d. physiol. Gesellsch. zu Berlin*, June 8, 1879.

[5] *Arch. f. Heilk.* vi. 286; viii. 343; x. 329.

[6] *Zeit. physiol. Chem.* iii. 410.

[7] *Arch. f. Anat. u. Physiol.* 1870, p. 159.

[8] *Med. Chir. Trans.* xlii. 361.

What is known as the epicritical increase of urea[1] is the greatly increased secretion of urea that occurs at the commencement of the defervescence of a fever. It is probably not due to an increased formation of urea, but to the removal of urea which has accumulated, owing to the fact that the kidneys have been acting sluggishly during the height of the fever.

Increased output of carbonic acid in fever was shown to exist in guinea-pigs by Pflüger and Colasanti (*see* pp. 373, 374), in dogs by Fränkel,[2] in men by Liebermeister and others.[3]

Other changes noted in fever are a rapid loss of the liver glycogen, a lessening of chlorides in the urine (*see* p. 760), and the appearance of pathological instead of normal urobilin in the urine (*see* p. 750).

The following table illustrates exchange of material in fever, no food being taken (Burdon-Sanderson[4]) :—

Income			Expenditure		
Disintegration of tissue	Nitrogen	Carbon	Excretions	Nitrogen	Carbon
Proteid, 120 gr.	18·6	63·6	Urea and uric acid, 40 gr. . . .	18·6	8·3
Fat, 205·7 gr. .	0·0	157·4	Respiration (CO_2) 780 gr. . . .	0·0	212·7
	18·6	221·0		18·6	221·0

This table should be compared with that on p. 834.

Diabetes mellitus.—In addition to the presence of sugar in the urine in this disease, the most marked symptoms are intense thirst and ravenous hunger. As a rule, diabetic patients digest their food well. The thirst is an indication of the necessity of replacing the large quantities of water lost by the kidneys ; the hunger, that of replacing the great waste of tissues that occurs. For not only does the urine contain sugar, but, in addition, a great excess of urea and uric acid. The carbonic acid output is somewhat smaller than in health. In health the carbohydrates, after assimilation, give rise, by oxidation, to carbonic acid ; in diabetes, all the carbohydrates do not undergo this change, but pass as sugar into the urine. Not that all the sugar of the urine is derived from carbohydrates, for many diabetics continue to pass large quantities when all carbohydrate food is withheld ; under these circumstances it must be derived from the destruction of proteid matter.

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[1] Cohnheim's *Pathology*, 2nd German edit. vol. ii. p. 582.

[2] *Arch. pathol. Anat.* lxxvi. 136.

[3] For references, *see* Senator, *Unters. ü. d. fieberhaften Process und seine Behandlung*, Berlin, 1873.

[4] *Practitioner*, April, May, June, 1876.

In spite of abundant nourishment, the body of a diabetic patient wastes; there is not equilibrium; the output is in excess of the intake. This is especially marked in the case of the excretion of nitrogen.[1]

Leucocythæmia.—This appears to be the only other disease in which systematic observations have been made on metabolic exchanges. Although, as in all chronic and debilitating diseases, there is a general loss of vitality, and a corresponding lessening of metabolic change, observers have failed to find anything very special or characteristic in this disease. This is somewhat remarkable, as so many important parts of the body may be effected, especially the blood and the blood-forming organs. There is, however, always an increase of uric acid in the urine.[2] With regard to urea the statements of various investigators are contradictory [3]

Luxus Consumption

In former portions of this book we have insisted on the fact that the food does not undergo combustion, or katabolic changes, until after it is assimilated, that is, until after it has become an integral part of the tissues. Formerly the blood was supposed to be the seat of oxidation; but the reasons why this view is not held now have been already given. When a student is first confronted with balance-sheets, representing metabolic exchanges, it is at first a little difficult for him to grasp the fact, that although the amount of nitrogen and carbon ingested is equal to the amount of the same elements which are eliminated, yet the eliminated carbon and hydrogen are not derived from the food direct, but from the tissues; the food becomes assimilated, and takes the place of the tissues thus disintegrated. Again, let us suppose the food to be increased in quantity; the excretions are also increased. Here the increased intake of food stimulates the tissues to increased combustion, pushing, as it were, the old out of the way to make room for the new. Let us suppose we have a tube open at both ends and filled with a row of marbles: if an extra marble is pushed in at one end, a marble falls out at the other: if two marbles are introduced instead of one, there is an output of two at the other end; if a dozen, or any larger number be substituted, there is always a corresponding exit of the same number at the other end of the tube; the marbles that fall out are equal in number to those put in at the other end, but the marbles that fall out are different ones from those put in. This very rough illustration may perhaps assist in the comprehension of the metabolic exchanges.

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[1] The following references to the chief papers on metabolism in diabetes are taken from Hoppe-Seyler's *Physiol. Chem.* p. 971: M. Traube, *Virchow's Archiv*, vol. iv.; Seegen, *Wiener med. Wochensch.* 1863, No. 14; also *Der Diabetes mellitus*, Berlin, 1875; F. Nasse, *Arch. f. physiol. Heilk.* 1851, p. 52; Reich, *Diss.* Greifswald, 1859; Rosenstein, *Virchow's Archiv*, xii. 414; C. Gähtgens, *U. d. Stoffwechsel eines Diabetikers*, Diss. Dorpat, 1866; E. Külz, *Beiträge zur Pathol. u. Therapie d. Diab. mell.*, Marburg, 1874–5; *Arch. f. experim. Pathol.* vi. 140; Pettenkofer and Voit, *Sitzungsb. Bayer. Akad.* November 1865; *Zeit. Biol.* iii. 380; C. Schmidt, *Charakteristik der epid. Cholera*, Leipzig and Mitau, 1850, p. 160; v. Mering, *Deutsch. Zeit. f. prakt. Med.* 1877, No. 18.

[2] The following references are again derived from Hoppe-Seyler's Text-book: *Virchow's Arch. f. path. Anat.* v. 108; H. Ranke, *Beobachtungen und Versuche ü. d. Ausscheidung d. Harnsaüre*, München, 1858; Pettenkofer and Voit, *Zeit. Biol.* v. 326; E. Salkowski, *Virchow's Archiv*, l. 174, lii. 58; K. B. Hofmann, *Wien. med. Woch.* 1870, Nos. 42, 43, 44; Schmutziger, Mosler, Fleischer, and Penzoldt, *Deutsch. Arch. f. klin. Med.* vol. xxvi. 1880, p. 1. All the foregoing agree in the statement that the output of uric acid is greater than normal.

[3] Salkowski states there is a lessening, Mosler an increase, Pettenkofer and Voit, Fleischer and Penzoldt, say there is neither increase nor decrease.

The difficulty just alluded to, which a student feels, was also felt by the physiologists who first studied metabolism; and Voit [1] formulated a theory, of which the following is the gist: All proteid taken into the alimentary canal appears to affect proteid metabolism in two ways; on the one hand, it excites rapid disintegration of proteids, giving rise to an immediate increase of urea; on the other hand, it serves to maintain the more regular proteid metabolism continually taking place in the body, and so contributes to the normal regular discharge of urea. He, therefore, supposed that the proteid which plays the first of these two parts is not really built up into the tissues, does not become living tissue, but undergoes the changes that give rise to urea, somewhere outside the actual living substance. Consequently, he divided the proteids into 'tissue-proteids,' which are actually built up into living substance, and 'floating or circulating proteids,' which are not thus built up, but by their metabolism outside the living substance set free energy in the form of heat only. It was at this time erroneously supposed that the exclusive use of proteid food was to supply proteid tissue elements, and that vital manifestations other than heat had their origin in proteid metabolism, the metabolism of fats and carbohydrates giving rise to heat only. Hence, when it was first surmised that a certain proportion of proteids underwent metabolism which gave rise to heat only, this appeared to be a wasteful expenditure of precious material, and the metabolism of this portion of food was spoken of as a 'luxus consumption,' a wasteful consumption. There were many deductions from this general theory to explain particular points; of these two may be mentioned: (1) In inanition, the urea discharged for the first few days is much greater than it is subsequently: this was supposed to be due to the fact that in the first few days all the floating capital was consumed ; (2) the effect of feeding with a mixture of gelatine and proteid (*see* p. 838) was supposed to be due to the fact that gelatin was able to replace 'floating proteid,' but not 'tissue proteid.'

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[1] *Zeit. Biol.* x. 221.

This theory of Voit's, ingenious and plausible at first sight, has met with but little general acceptance, because so many observed facts are incompatible with it, and I cannot do better than conclude this chapter by giving the opinions of three eminent physiologists on the question.

Professor Michael Foster[1] writes as follows : 'The evidence we have tends to show that in muscle (taking it as an instance of a tissue) there exists a framework of what we may call more distinctly living substance, whose metabolism, though high in quality, does not give rise to massive discharges of energy, and that the interstices, so to speak, of this framework are occupied by various kinds of material related in different degrees to this framework, and therefore deserving to be spoken of as more or less living, the chief part of the energy set free coming directly from the metabolism of some or other of this material. Both framework and intercalated material undergo metabolism, and have in different degrees their anabolic and katabolic changes ; both are concerned in the life of the organism, but one more directly than the other. We can, moreover, recognise no sharp break between the intercalated material and the lymph which bathes it ; hence such phrases as "tissue proteid" and "floating proteid" are undesirable if they are understood to imply a sharp line of demarcation between the "tissue" and the blood or lymph, though useful as indicating two different lines or degrees of metabolism.'

Professor Burdon-Sanderson[2] writes as follows : 'The production of urea and other nitrogenous metabolites is exclusively a function of "living material" ; and this process is carried on in the organism with an activity which is dependent on the activity of the living substance itself, and on the quantity of material supplied to it. No evidence at present exists in favour of a "luxus consumption" of proteid.'

Professor Hoppe-Seyler,[3] after stating that he can make out no clear distinction between the two varieties of proteid from Voit's own writings, proceeds as follows: 'Voit states that the circulating proteid is no other than that which is dissolved in the tissue juice, which is derived from the lymph-stream, and ultimately from the circulating blood. He (Voit) further says: "As soon as the proteid of the blood-plasma leaves the blood-vessels, and circulates among the tissue elements themselves, it is then the proteid of the nutrient fluid or circulating proteid. It is no longer proteid of the blood-plasma, nor yet is it the proteid of the lymph-stream." The place where Voit situates his circulating proteid is beyond the ken of the anatomist; it is in a mysterious space between tissue-elements, blood-vessels, and lymph-vessels; the chemist meets with equal difficulties, as there is apparently no chemical difference between tissue proteid and circulating proteid. I can, therefore, arrive at no other conclusion than that these terms are not only useless, but unscientific, and are the outcome of speculations in a region where there is as yet no positive knowledge. These criticisms on Voit's theories do not, however, by any means, lessen the importance and high value of the immense amount of practical research carried on by Voit and his pupils.'

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[1] *Text-book*, 5th edit. pp. 825, 826.

[2] *Syllabus of Lectures*, p. 37.

[3] *Physiol. Chem.* p. 974.

I have placed Professor Foster's view first because it takes into account certain facts which tend to show that there are degrees in metabolism. The most important of these seems to be the formation of amido-acids in the intestine. The fate of tyrosine is uncertain; but it is an undoubted fact that by feeding an animal on leucine, the urea is increased. The transformation of leucine into urea occurs in the liver. It can hardly be supposed that leucine becomes to any great extent an integral part of the living framework of the liver cells, but like other extractives, and like aromatic compounds absorbed from the alimentary canal, it becomes a part of what Foster terms the intercalated material. Here it undergoes the final change, and is ultimately and apparently very rapidly discharged in the urine. Dr. Sheridan Lea[1] has recently discussed in a very able manner the probable *rôle* of the amido-acids in the animal economy, and he compares it to the part played by the salts of the food. Neither salts nor extractives simply pass into the urine without fulfilling a useful purpose on their way; but the exact and specific use of each, whether on the synthetic or analytic side of metabolic phenomena, must be the subject of renewed research.

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[1] *Journ. of Physiol.* vol. xi. 262.

CHAPTER XLVIII

ANIMAL HEAT

Among the most important results of the chemical processes we sum up under the term metabolism, is that of the production of heat. Heat, like mechanical work, is the result of the katabolic side of metabolic processes ; the result, or accompaniment, that is to say, of the formation of carbonic acid, water, urea, and other excreted products.

As regards temperature, animals may be divided into two great classes :—

(1) Warm-blooded or homoiothermal animals, or those which have an almost constant temperature. This class includes mammals and birds.

(2) Cold-blooded or poikilothermal animals, or those whose temperature varies with that of the surrounding medium, being always, however, a degree, or a fraction of a degree, above that of the medium. This class includes reptiles, amphibians, fish, and probably all invertebrates.

The temperature of a man in health varies but slightly, being between 36·5° and 37·5° C. (98° to 99° F.) Most mammals have approximately the same temperature : horse, donkey, ox, 37·5° to 38° ; dog, cat, 38·5° to 39° ; sheep, rabbit, 38° to 39·5° ; mouse, 40° C. Birds have a higher temperature, about 42° C. The temperature varies a little in different parts of the body, that of the interior being greater than that of the surface ; the blood coming from the liver when oxidation is very active is warmer than that of the general circulation ; the blood becomes rather cooler in its passage through the lungs.

The temperature also shows slight diurnal variations, reaching a maximum about 3 P.M. (37·5° C.) and a minimum about 3 A.M. (36·8° C.); that is, at a time when the functions of the body are least active. If, however, the habits of a man be altered, and he sleeps in the day, working during the night, the times of the maximum and minimum temperatures are also inverted. Inanition causes the temperature to fall, and just at the onset of death it may be below 30° C. Active muscular exercise raises the temperature temporarily by about 0·5° to 1° C. Diseases may cause the temperature to vary considerably, especially those which we term febrile (*see* p. 841).

Although certain mechanical actions, such as friction, due to movements of various kinds, may contribute a minute share in the production of heat in the body, yet we have no knowledge as to the actual amount thus generated. The great source of heat is, as already stated, chemical action, especially oxidation. Any given oxidation will always produce the same amount of heat. Thus, if we oxidise a gramme of carbon, a known amount of heat is produced, whether the element be free or in a chemical compound. The following figures show the approximate number of heat-units produced by the combustion of one gramme of the following substances. A heat-unit, or calorie, is the amount of heat necessary to raise the temperature of one gramme of water 1° C. (*see* also p. 605 *et seq.*) :—

Hydrogen	3450	Fat	9069
Carbon	8100	Cane sugar	3348
Urea	2205	Starch	3898
Albumin	4998		

The question of the heat-value of various foods has been already discussed (p. 606). It is, however, most important to remember that the 'physiological heat-value' of a food may be different from the 'physical heat-value,' i.e. the amount of heat produced by combustion in the body may be different from that produced when the same amount of the same food is burnt in a calorimeter. This is especially the case with the proteids, for they do not undergo complete combustion in the body, for each gramme of proteid yields a third of a gramme of urea, which has a considerable heat-value of its own. Thus albumin, which, by complete combustion, yields 4998 heat-units, has a physiological heat-value=4998 *minus* one-third of the heat-value of urea (2205)=4998 − 735=4263.

Of the heat produced in the body, it is estimated by Helmholtz that about 7 per cent. is represented by external mechanical work, and that of the remainder about four-fifths are discharged by radiation and evaporation from the skin, and the remaining fifth by the lungs and excreta.

The following table [1] exhibits the relation between the production and discharge of heat in twenty-four hours in the human organism at rest, estimated in calories.[2] The table conveniently takes the form of a balance-sheet in which production and discharge of heat are contrasted; to keep the body-temperature normal these must be equal. The basis of the table in the left-hand (income) side is the same as Ranke's adequate diet (*see* p. 604 and p. 832):—

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[1] *See* Dr. Sanderson's *Syllabus of Lectures*, p. 42.

[2] The calorie we are taking is sometimes called the small calorie; by some the word *caloric* is used to denote the amount of heat necessary to raise one kilogramme of water 1° C.

Production of heat		*Discharge of heat*	
Consumption of	Calories		Calories
Proteid (100 gr.) .	100 × 4263 = 426,300	Warming water in food, 2·6 kilos. × 25° C. =	65,000
Fat (100 gr.) . .	100 × 9069 = 906,900	Warming air in respiration, 16 kilos. × 25° × 0·24 =	96,000
Carbohydrates (250 gr.)	250 × 3898 = 974,500	Evaporation in lungs, 630 gr. × 582 =	366,660
		Radiation and evaporation at surface, =	1,780,040
	2,307,700		2,307,700

The figures under the heading Production are obtained by multiplying the weight of food by its physiological heat-value. The figures on the other side of the balance-sheet are obtained as follows: The water in the food is reckoned as weighing 2·6 kilos. This is supposed to be at the temperature of the air taken as 12° C.; it has to be raised to the temperature of the body, 37° C., that is through 25° C. Hence the weight of water multiplied by 25 gives the number of calories expended in heating it. The weight of air is taken as weighing 16 kilos.; this also has to be raised 25° C., and so to be multiplied by 25; it has further to be multiplied by the relative heat of air (0·24). The 630 grammes of water evaporated in the lungs has to be multiplied by the potential or latent heat of steam at 37° C. (582). The portion of heat lost by radiation and evaporation from the skin constitutes about four-fifths of the whole, and is obtained by deducting the three previous amounts from the total.

This table does not take into account the small quantities of heat lost with urine and fæces. It need hardly be remarked that the above is a mere illustrative experiment. Changes in the diet, in the atmospheric temperature, in the temperature of the food taken, in the activity of the sweat-glands, in the amount of moisture in the atmosphere, and in the amount of work done would considerably alter the above figures.

Calorimetry.—Calorimeters employed in chemical operations are not suitable for experiments on living animals. An animal surrounded by ice or mercury, the melting and expansion of which respectively are measures of the amount of heat evolved, would be under such abnormal conditions that the results would be valueless.

The apparatus most usually employed is the water calorimeter. This was first used by Lavoisier, and his apparatus as modified by Dulong is shown in fig. 104. The animal is placed in a metal chamber, surrounded by a water-jacket. There are tubes for the entrance and exit of the inspired and expired gases respectively. The heat given out by the animal warms the water in the jacket, and is measured by the rise of temperature observed in the water, of which the volume is also known. The air which passes out from the chamber goes through a long spiral tube, passing through the water-jacket, and thus the heat is abstracted from it and not lost.

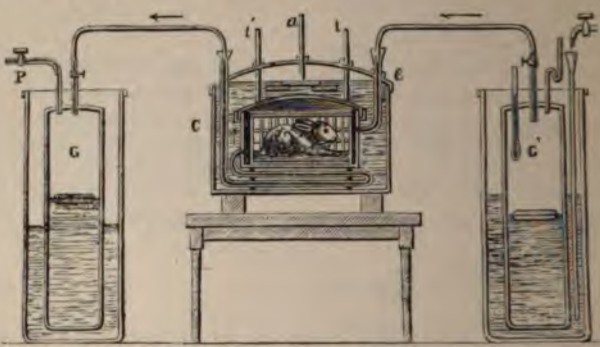

FIG. 104.—Dulong's Calorimeter: C, calorimeter, consisting of a vessel of cold water in which the chamber holding the animal is placed; G′, gasometer from which air is expelled by a stream of water. The air enters the respiratory chamber. G, gasometer receiving the gases of expiration and the excess of air. *t*, *t′*, thermometers; *a*, a wheel for agitating the water. Observe the delivery-tube on the left is much twisted in the water-chamber, so as to give off its heat to the surrounding water. (From McKendrick's 'Physiology.')

Rosenthal[1] has invented an air-calorimeter, in which an air-jacket takes the place of the water-jacket of Dulong's apparatus.

Regulation of the Temperature of Warm-blooded Animals

We have seen that heat is produced by combustion processes, and lost in various ways. In order to maintain a normal temperature, both sides of the balance-sheet must be equal. This equalisation may be produced by the production of heat, adapting itself to variations in discharge, or by the discharge of heat adapting itself to variations in production, or lastly, and more probably, both sets of processes may adapt themselves mutually to one another. We have, therefore, to consider (1) regulation by variations in loss and (2) regulation by variations in production. The following is a *résumé* of our knowledge on these two points, as given in Professor Foster's text-book.[2]

Regulation by variations in loss.—The two means of loss susceptible of any amount of variation are the lungs and the skin. The more air that passes in and out of the lungs, the greater will be the loss in warming the expired air and in evaporating the water of respiration. In such animals as the dog, which perspire but little, respiration is a most important means of regulating the temperature; and in these animals a close connection is observed between the production of heat and the respiratory activity.[1] The great regulator, however, is undoubtedly the skin, and this has a double action. In the first place, it regulates the loss of heat by its vaso-motor mechanism ; the more blood passing through the skin, the greater will be the loss of heat by conduction, radiation, and evaporation. Conversely, the loss of heat is diminished by anything that lessens the amount of blood in the skin, such as constriction of the cutaneous vessels, or dilatation of the splanchnic vascular area. In the second place, the special nerves of sweat-glands are called into action. Familiar instances of the combined action of these two sets of nerves are the reddening of the skin and sweating that occur after severe exercise, on a hot day, or in a hot-air or vapour bath, and the pallor of the skin and absence of sensible perspiration on the application of cold to the body.

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[1] *Arch. f. Physiol. u. Anat.; physiol. Abth.* 1889, p. 1.

[2] P. 810 *et seq.*

Regulation by variations in production.—The rate of production of heat in a living body, as determined by calorimetry, depends on a variety of circumstances. It varies in different kinds of animals. The general rate of katabolism of a man is greater than that of a dog, and of a dog greater than that of a rabbit. Probably every species has a specific coefficient, and every individual a personal coefficient of heat-production, which is the expression of the inborn qualities proper to the living substance of the species and individual. Another factor is the proportion of the bulk of the animal to its surface area, the struggle for existence raising the specific coefficient of the animals in which the ratio is high. Other important considerations are the relation of the intake of food to metabolic processes, and the amount of muscular work which is performed. These various influences are themselves regulated by the nervous system, and physiologists have long suspected that afferent impulses arising in the skin or elsewhere may, through the central nervous system, originate efferent impulses, the effect of which would be to increase or diminish the metabolism of the muscles and other organs, and by that means increase or diminish respectively the amount of heat there generated. That such a metabolic or thermogenic nervous mechanism does exist in warm-blooded animals is supported by the following experimental evidence :—

(1) Though in cold-blooded animals, a rise or fall of the surrounding temperature causes respectively a rise and fall of their metabolic activity, in a warm-blooded animal the effect is just the reverse. Warmth from the exterior demands a diminished production of heat in the interior, and *vice versâ*.

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[1] The panting of a dog when overheated is a familiar instance of this. A dog also, under the same circumstances, puts out its tongue, and loses heat from the evaporation that occurs from its surface.

(2) That this is due to a reflex nervous impulse is supported by the fact that a warm-blooded animal, when poisoned by curare, no longer manifests its normal behaviour to external heat and cold, but is affected in the same way as a cold-blooded animal. Section of the medulla produces the same effects, as the nerve-channels, by which the impulses travel, are severed. When curare is given, the reflex chain is broken at its muscular end, the poison exerting its influence on the end-plates, and causing a diminution of the chemical tonus of the muscles. The centre of this thermotaxic reflex mechanism must be situated somewhere above the spinal cord.

(3) Various injuries caused by accident, or purposely produced by puncture, or cautery, or electrical stimulation of limited portions of the more central portions of the brain, may give rise to great increase of temperature, not accompanied by other marked symptoms.[1]

We thus see that the nervous system is intimately associated with the regulation of the temperature of the body. There is at least one —there may be several centres associated in this action. The centres receive afferent impulses from without; they send out efferent impulses by at least three sets of nerves: (1) the vaso-motor nerves, (2) the secretory nerves of the sweat-glands, (3) trophic or nutritional nerves. The first two sets of nerves, the vaso-motor and the secretory, affect the regulation of temperature on the side of discharge; the third set of nerves may be special nerve-fibres set apart for the regulation of chemical processes in the organs they supply; or it may be that all nerves to muscles and other organs are capable of transmitting trophic impulses. The discussion as to whether there are or are not special trophic fibres is an interesting one; but in whichever way this is finally settled it does not matter in the least in the present consideration. The fact remains that this third set of nerve-impulses affects the regulation of temperature on the side of production. Gaskell has gone so far as to consider that the impulses may be of two kinds: the nerves which convey those impulses which produce building up of tissue, or anabolism, he calls *anabolic nerves*, while those that bring about the reverse process he terms *katabolic nerves*.

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[1] *See* a recent paper by Hale White, *Journ. of Physiol.* ii. 1.

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